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SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

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ANNUAL CONVENTION—AMERICAN PHARMACEUTICAL ASSOCIATION
DENVER, COLORADO, AUGUST, 1942

Published by the American Pharmaceutical Association. Publication office: 20th and Northampton Sts., Easton, Pa. Editorial office: 2215 Constitution Ave., Washington, D. C.

Annual Subscription: Journal of the American Pharmaceutical Association, complete $5.00; Canada, $6.00; Other Foreign, $6.50. (a) Scientific Edition, $4.00; Canada, $4.35; Other Foreign, $4.50; Single Numbers, $0.35; Canada, $0.40; Other Foreign, $0.50. (b) Practical Pharmacy Edition, $1.50; Canada, $1.70; Other Foreign, $2.00; Single Numbers, $0.15; Canada, $0.20; Other Foreign, $0.25.

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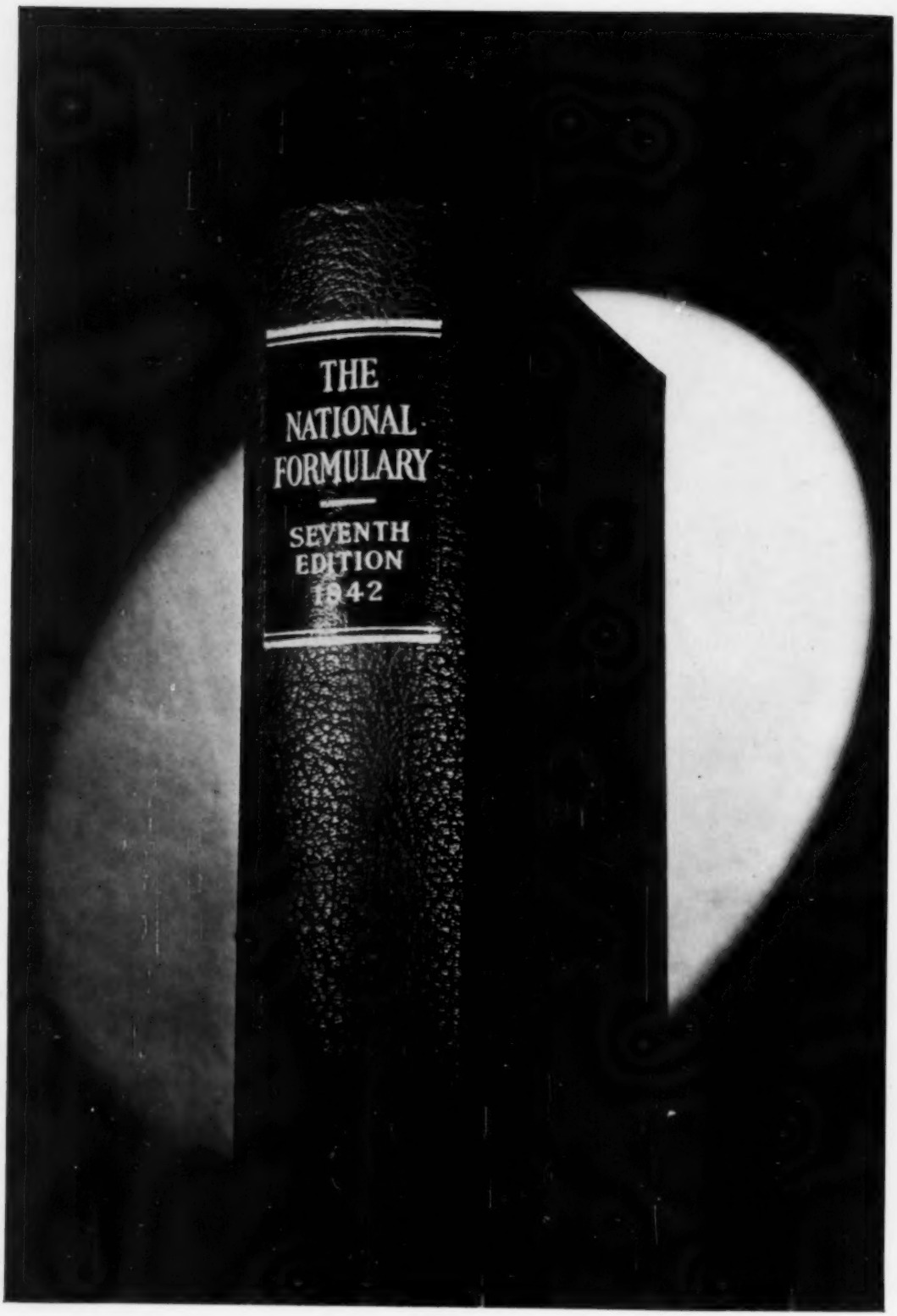
Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year: Scientific Edition monthly on the fifth. Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

Vol. XXXI, No. 7 JULY, 1942


THE
NATIONAL
FORMULARY
SEVENTH
EDITION
1942

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXI | *JULY, 1942* | NUMBER 7 CONSECUTIVE NO. 13

Chemical Examination of the Seeds of *Barringtonia acutangula* Gaertn.*

By J. K. Lahiri and S. Ghosh

Barringtonia acutangula Gaertn. (*Myrtaceæ*) is one of the most widely distributed trees in Bengal and is known in Bengali as "Hijjal." The fruit is known as "nurse fruit" and is one of the best-known domestic remedies. When children suffer from cold in the chest, the seed is rubbed down with water on a stone and applied over the sternum. A few grains, administered internally, seldom fails to induce vomiting which results in expulsion of mucus from the air passages. The seed is also used to reduce enlarged abdomens in children (1).

The seeds, leaves and roots are stated to act as a fish poison.

No chemical or pharmacological investigation appears to have been made of the constituents of the seeds of *B. acutangula* growing so widely in Bengal. The present investigation consists of an attempt to elucidate the chemical composition of the seeds.

Greshoff (2) and Weil (3) have reported the presence of saponin-like glycosides in *B. insignis*, *B. vriesei* and *B. racemosa*. Van den Driessen-Mareeuw (4) has isolated a saponin, barringtonin, from the seeds of *B. spinosa* and Nozoe (5) has also isolated a saponin, barrinin, from the seeds of *B. asiatica*.

The fresh fruits of *B. acutangula* were collected from the Dacca district during the season when they were fully grown. The seeds obtained from them were air dried and powdered.

EXPERIMENTAL

Preliminary Examination.—The ash content of the air-dried and powdered seeds was found to be 4.85%. A hot water infusion gave a filtrate acid to litmus. The infusion gave on shaking the characteristic honeycomb froth indicating the presence of saponins. Steam distillation did not indicate volatile constituents. Extraction with Prollius' fluid and with weak hydrochloric acid gave negative results with the usual alkaloidal reagents (6).

Extraction with Selective Solvents.—Air-dried and powdered seeds were extracted in a Soxhlet extractor with selective solvents. Petroleum ether (b. p., 40–60° C.) extracted 2.0%, ether 5.5% and rectified spirit 4.1%. The petroleum ether extract showed the presence of an oil and traces of a sterol. The ether extract showed the presence of a substance which gave a blue-black precipitate with ferric chloride. The rectified spirit extract showed the presence of a bitter saponin. The marc left after extracting with the above solvents was extracted several times with 45% alcohol. The combined aqueous alcoholic extracts were concentrated and treated with lead acetate. The lead acetate precipitate also showed the presence of saponins.

* From the Department of Chemistry, School of Tropical Medicine, Calcutta, India.

Isolation of the Saponin.—Air-dried and powdered seeds (200 Gm.) were refluxed four times with 45% alcohol. The combined extracts were concentrated to a small bulk, filtered and treated with an aqueous solution of lead acetate. The precipitate obtained was suspended in 75% alcohol and the lead was removed by a current of hydrogen sulfide. The filtrate was concentrated and a little alcohol added. The precipitate formed, consisting of starchy matter, was filtered off and rejected. Further amounts of alcohol were added and the first lot of crude saponin (0.81 Gm.) precipitated was filtered off. Ether was added to the alcoholic filtrate and the second crop of the crude saponin (0.75 Gm.) was obtained. On drying over concentrated sulfuric acid in vacuum the saponin was obtained as a yellow powder (yield 0.78%). The crude saponin was purified by dissolving it in the smallest quantity of water and precipitating it with alcohol. The flocculent precipitate formed was collected and dried over concentrated sulfuric acid and was obtained as a white amorphous powder. Addition of ether to the alcoholic filtrate gave a precipitate which on drying formed a yellow amorphous powder. The white and the yellow powders gave all the tests for saponin. The white powder was further purified by dissolving it in smallest quantity of water and precipitation by alcohol. The saponin was thus obtained as a white amorphous powder which decomposed at 148° C.

The filtrate obtained after removing the lead acetate precipitate was treated with lead subacetate but nothing of interest could be obtained either from the lead subacetate precipitate or the filtrate.

The Saponin.—The white amorphous saponin, acid in nature, was very soluble in water and insoluble in petroleum ether, ether, chloroform and alcohol. On hydrolysis the saponin gave sugars. Alcoholic cholesterol and baryta water gave a white precipitate with the saponin.

Pharmacological Action.[1]—The saponin possessed strong hæmolytic action and was found to kill fish. *Macrones vittatus* were experimented with and the opercular movement of the fish, kept under water, was recorded on a kymograph before and after the addition of the saponin.

SUMMARY

The toxic principle of the seeds of *Barringtonia acutangula* is an amorphous acid saponin. Other substances such as starch, fatty oil and sugars are present. The poisonous action of the seeds on fish could be accounted for by the presence of the saponin.

[1] The authors desire to express their thanks to Dr. N. N. Das for carrying out the pharmacological experiments.

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Interrelated Vitamin Requirements

The Influence of Thiamin, Riboflavin, Pantothenic Acid and Vitamin B_6 on Liver Glycogen Reserves*

By G. C. Supplee, R. C. Bender and Z. M. Hanford

The degree and rate of glycogen formation and hydrolysis in the liver are influenced by many factors, including dietary components, energy demands of the organism, state of metabolism and the degree of normality of glandular secretions. As yet, only fragmentary and incidental data concerning the influence of vitamin entities are available, notwithstanding much evidence of an inferential character indicating that important reactions involving these factors are largely localized in the liver. Although glycogenesis and glycogenolysis are recognized as liver functions, the chemical reactions involved are only generally known to be of an oxidative or reducing character, and it has not been determined to what degree individual vitamin entities may be directly or indirectly concerned. The investigations reported herein were designed to determine the interrelationship and effect of each of four entities of the vitamin B complex on glycogen formation

* From the Borden Company Biological and Chemical Research Laboratories, Bainbridge, N. Y.

and retention in the liver. The controls and techniques adopted for the study, as will be described hereinafter, are believed to be an improvement over methods frequently employed.

EXPERIMENTAL

The investigation involved the use of about 600 white rats and extended over a period of about sixteen months. The procedure involved the depletion of a large number of animals of each of the vitamins to be tested. Following depletion, a 24-hr. starvation period was imposed prior to administration of the test substance. Forced feeding with a blunt hypodermic needle used as a stomach tube, of 1 Gm. of the basal ration and vitamin supplements other than the particular one under investigation was the common practice for the controls and test animals immediately following the 24-hr. fast period. (The basal ration with a fluidity suitable for handling in this manner was prepared by mixing 50 Gm. of the dry product with sufficient water to make a 60-ml. volume.) The vitamins tested were thiamin, riboflavin, pantothenic acid and vitamin B_6. These were administered in 100-γ quantities (with exceptions noted hereinafter) in 0.5 ml. of physiological salt solution by injection directly into the heart. This method of administration obviates loss through excretion, variable absorption and other uncontrolled features inherent in oral feeding or injection at other sites. Less than 2% of casualties have resulted at the hands of experienced operators during the injection of several hundred animals involved in this and other studies.

Animals properly depleted of each substance were divided into two major groups with an equal number of each sex. One group served as negative controls which received the 1 Gm. of food only; the other group received both the food and the injected vitamin. From 8 to 12 animals were immediately sacrificed and the liver removed for analysis. The remaining animals in each group were returned to their respective cages and supplied with water only. At 4-hr. intervals throughout a 24-hr. period, not less than 4 and sometimes as many as 8 animals from both the control and injected groups were sacrificed and liver glycogen determined. Glycogen determinations were carried out according to the method of Good, Kramer and Somogyi (1).

The depletion of the animals preparatory to determining the influence of individual vitamin factors was as follows: White rats, 23 to 25 days old, were placed in individual screened bottom cages and supplied the following basal ration: vitamin-free casein,[1] 20 parts; sucrose, 69 parts; hydrogenated vegetable oil,[2] 3 parts; salt mixture No. 40,[3] 4 parts; powdered agar-agar, 2 parts; and cod liver oil (medicinal grade), 2 parts. At the end of one week, the requisite primary supplements were supplied as a dosage per rat per day.

Animals to be depleted of thiamin were supplied with 10 γ of riboflavin and 100 mg. of rice polish concentrate[4] autoclaved at *p*H 8.5 for five hours at 120° C. This autoclaved product contains no vitamin B_1 or riboflavin but does contain from 11 to 13 γ of pantothenic acid, and 7 to 8 γ of vitamin B_6 per 100 mg. following the heat treatment. The criteria for determining uniform depletion were constant or declining weight and manifestation of "medium" paralytic symptoms.

Animals to be depleted of riboflavin were supplied with 12.5 γ of thiamin and 100 mg. of alkaline (*p*H 8.5) autoclaved rice polish concentrate. The criterion of depletion was constant weight or slow growth not exceeding about 2 Gm. per week; this state of depletion was reached after six to eight weeks.

Animals to be depleted of vitamin B_6 were supplied with 12.5 γ of thiamin, 10 γ of riboflavin and 100 mg. of rice polish factor II.[5] This product contains no vitamin B_1, riboflavin or vitamin B_6 (2), but does contain approximately 20 γ of pantothenic acid per 100 mg. The criteria of depletion were cessation of growth and development of the acrodynia type dermatitis, usually manifested in five to seven weeks.

The animals to be depleted of pantothenic acid were supplied 12.5 γ of thiamin, 10 γ of riboflavin, 10 γ of vitamin B_6 and 100 mg. of the rice polish factor II autoclaved at *p*H 11 for five hours at 120° C. This alkaline heat-treated product contains 0.3 γ or less of pantothenic acid per 100 mg., but no riboflavin, thiamin or vitamin B_6. Evidence of depletion was slow growth or constant weight and occasional sudden and unpredictable deaths among the group; these manifestations usually occurred within six to eight weeks and pantothenic acid depletion could frequently be confirmed by evidence of adrenal and/or kidney damage upon autopsy (3, 4).

Certain variations in these procedures were carried out with other groups of animals in which it was desired to determine the effect of the absence of all of the factors or variations in the amount of a particular factor on glycogen formation and retention; specifically, a comparison of the influence of 25-γ and 100-γ injections of riboflavin, the influence of previous supplements of pantothenic acid, and the influence of previous saturation of the tissues with thiamin. The only departures from the basic plan of procedure and techniques required for these particular studies were: in the riboflavin comparison, the injection of 25 γ in lieu of 100 γ; for the pantothenic acid variation, a daily supplement of 50 γ of pantothenic acid in addition to the other primary supplements was provided; and for the study involving tissue saturation with thiamin, the animals were first depleted to the paralytic state

[1] Labco brand. [2] Crisco. [3] Steenbock, H., and Nelson, E. M., *J. Biol. Chem.*, 56 (1923), 355.

[4] Labco brand. [5] Labco brand.

and then supplied with 100 γ of thiamin per day for five days in addition to the other primary supplements. All groups of animals prepared according to these particular requirements were subjected to the usual 24-hr. starvation period prior to the forced feeding of 1 Gm. of the basal ration and supplements and injection of the test substance.

The results from the entire study are shown in the accompanying area graphs in Figs. 1 to 4. A higher liver glycogen level at specified periods up to 24 hrs. resulted from all vitamin injections with the possible exception of the group which received a simultaneous injection of all four factors (Fig. 1, group I). The period of impoverishment during which the animals in this group could be maintained without any of the supplementing factors was only three weeks. Since it is well established that experimental animals cannot be depleted of any one of these vitamins in a 3-week period if the other factors are supplied, it is reasonable to assume that a substantial residuum of body stores existed in the impoverished animals to a degree which did not permit the variations shown by the groups more fully depleted of a single factor. All such groups showed characteristic glycogen storage patterns with variations attributable to the particular vitamin factor tested. These variations are essentially of the same character irrespective of whether the glycogen is compared on the basis of the total amount in the entire liver, or as the concentration per Gm. of water-free liver tissue. While the data are presented as average values, the relationship between animals in comparable control and test groups was in no case found to be in opposition to the average of the relationships shown in the graphs

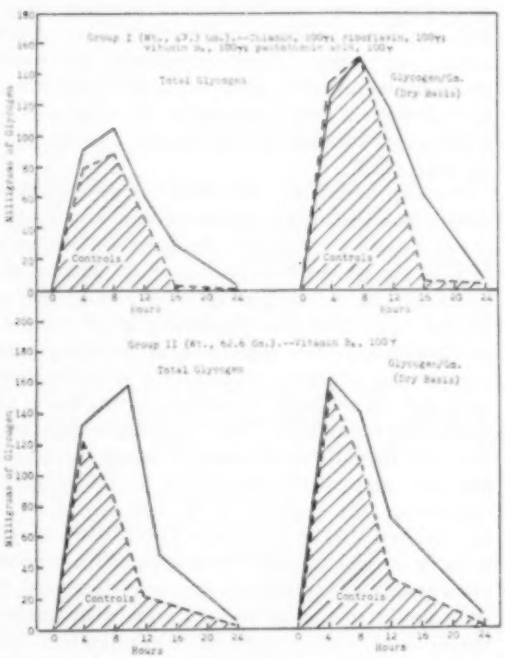

Fig. 1.—Liver Glycogen as Affected by Vitamin Supplements.

An examination of the data from the animals receiving the 100-γ and 25-γ injections of riboflavin indicates (Fig. 2) that the influence of this factor upon glycogen storage in the liver is relatively less than the influence of the other factors. Complete tissue depletion of riboflavin has never been reported and it is probable that such an accomplishment is impossible in the living animal from either a practical or theoretical point of view. Furthermore, unpublished data have shown that riboflavin is mobilized in the liver during digestion and assimilation even though body stores are greatly impoverished as a result of the absence of this factor in the dietary.

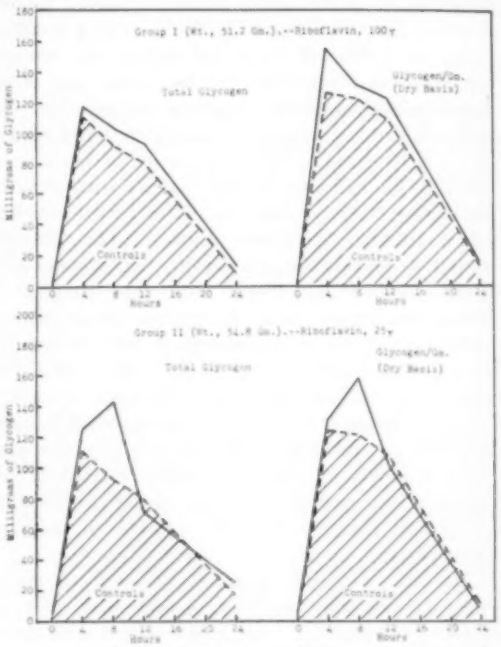

Fig. 2.—Liver Glycogen as Affected by Vitamin Supplements.

The data from the thiamin series (Fig. 3) show that the injection of 100 γ of this factor causes a marked increase in glycogen formation in depleted animals. The groups receiving 100 γ orally for five days following depletion in order to obtain a uniform and relatively high degree of tissue saturation prior to determining the effect of the 100-γ injection, did not show the same quantitative effect of the injection exhibited by the depleted group. This comparative evidence is interpreted as indicating a greater and more direct effect of thiamin deficiency on liver glycogen formation than results from riboflavin impoverishment.

The results from the pantothenic acid series shown in Fig. 4 present a still further variation in the liver glycogen pattern. There was not so great a variation in glycogen levels between the control and injected groups as prevailed in the thiamin series. This may be due to less complete exhaustion of body stores, or it may be due to a less significant

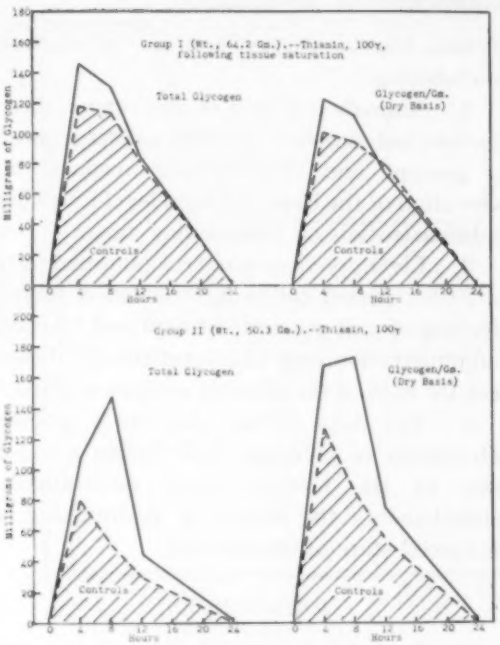

Fig. 3.—Liver Glycogen as Affected by Vitamin Supplements.

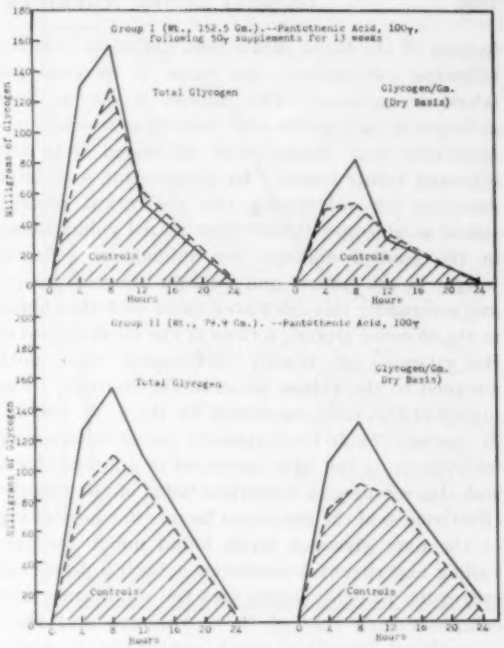

Fig. 4.—Liver Glycogen as Affected by Vitamin Supplements.

physiological role. It is significant, however, that the increase in liver glycogen caused by the injection of 100 γ is substantially of the same order of magnitude, irrespective of whether previous impoverishment was severe or only suboptimal.

The influence of vitamin B_6 impoverishment on the liver glycogen pattern (Fig. 1, group II) is characterized by a rapid decrease following the initial high level reached in a comparatively short time. Since an alteration of this pattern is brought about by injection of the vitamin, the results indicate that this factor may be involved in regulating the rate of glycogenolysis.

Since the present studies are believed to have been well controlled with respect to preparation of the animals, controlled intake of the supplementing and test substances, and uniform caloric intake during the 24-hr. test period, the data were further integrated in an attempt to evaluate the influence of each factor in terms of a comparable mathematical expression. The treatment of the data with this objective in view is briefly described as follows.

Various data showing the caloric requirements of white rats were reviewed for the purpose of selecting the value most applicable to the animals used and maintained under the conditions prevailing in these studies. The average basal metabolism rate of 744 calories per day per square meter of body surface as determined by Lewis and Luck (5) was selected as a suitable basis for the calculations. The caloric requirement per 4-hr. period for individual animals of given weight was readily determined by reference to the data reported by Diack (6). These calculated values varied from 1.11 calories for a 43-Gm. animal, the lowest in the thiamin series (Fig. 3, group II), to 2.85 calories for a 170-Gm. animal, the highest in the pantothenic acid series (Fig. 4, group I). Although the 4.11 calories provided by the 1-Gm.

TABLE I.—RATIO OF EFFECTIVENESS OF THIAMIN, RIBOFLAVIN, VITAMIN B_6 AND PANTOTHENIC ACID IN INFLUENCING LIVER GLYCOGEN LEVELS[a]

Vitamin Factor	Amount, γ	Figure Reference	Ratio of Effectiveness 4 Hrs.	8 Hrs.	12 Hrs.	16 Hrs.
Thiamin, riboflavin, vitamin B_6 and pantothenic acid	100 of each	1, group I	1:1.20	1:1.23	1:1.22	1:1.15
Vitamin B_6	100	1, group II	1:1.11	1:1.94	1:2.21	....
Riboflavin	100	2, group I	1:1.07	1:1.49	1:1.22	1:0.70
Riboflavin	25	2, group II	1:1.18	1:1.13	1:0.81	....
Thiamin	100[b]	3, group I	1:1.10	1:0.98	1:0.99	....
Thiamin	100	3, group II	1:1.32	1:3.29	1:1.45	....
Pantothenic acid	100[c]	4, group I	1:1.45	1:1.28	1:0.90	....
Pantothenic acid	100	4, group II	1:1.43	1:1.49	1:1.01	....

[a] Unity is assigned to the results from the controls.
[b] Following 5-day tissue saturation by oral feeding of 100 γ per day.
[c] Following oral feeding of 50 γ per day for 13 weeks.

feeding of the basal ration does not enter into the following calculations, the value is recorded for reference purposes. The calories stored as liver glycogen at each of the 4-hr. periods at which determinations were made, were calculated from the glycogen values found. By formulating a suitable equation for calculating the theoretical calories stored as glycogen (based upon actual values found in the control animals) by animals of different weight in the control and vitamin injected groups, and comparing this calculated value with that found in the injected groups, a ratio of the effectiveness of the vitamin was readily determined, with unity assigned to the values found in the controls. The utility of this ratio calculated for the 4-, 8- and 12-hr. periods (Table I) is primarily one of convenience for evaluating the data presented in graphical form and also serves as a numerical index of the relative effectiveness of the particular factors in contributing to the liver glycogen levels found under the prevailing experimental conditions. Such a numerical expression likewise emphasizes the significance and complementing role of these vitamins and their interrelated functions which contribute to maintenance of normal metabolism and efficiency of food utilization.

SUMMARY

1. Impoverishment of body stores of thiamin, riboflavin, vitamin B_6 and pantothenic acid resulting from inadequate dietary intake causes lowered liver glycogen levels indicating inefficient food utilization, deficient reserve energy and an unbalanced metabolism.

2. Animals depleted of any one of these factors but supplied with the others showed a prompt and characteristic response in elevation of the liver glycogen level following administration of the missing factor.

3. Liver glycogen patterns obtained over a 24-hr. period following controlled forced feeding of unit amounts of food and vitamin administration were characteristically different for each of the vitamin entities studied.

4. The data do not disclose a specific glycogenic or glycogen hydrolyzing role for any of the factors. Each undoubtedly complements the others in maintaining a balanced state of metabolism.

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Inhibiting Effect of Various Compounds on Curative Action of Sulfanilamide and Sulfapyridine in Infected Mice*

By George W. Raiziss, M. Severac and J. C. Moetsch

Lockwood (1, 2), Stamp (3), Green (4) and Fleming (5) contributed to the study of substances inhibiting the bacteriostatic effect of sulfanilamide. Woods and Fildes (6, 7) found that *p*-aminobenzoic acid present in yeast inhibits the bacteriostatic effect of sulfanilamide. Selbie (8) showed that *p*-aminobenzoic acid administered orally inhibits the therapeutic effect of sulfanilamide in mice infected with *Streptococcus hæmolyticus*.

EXPERIMENTAL

We studied the inhibiting effect *in vivo* of *p*-aminobenzoic acid on sulfanilamide and sulfapyridine administered to infected mice. Closely related products of *p*-aminobenzoic acid and other chemical compounds were included in this investigation.[1] Some mice were infected intraperitoneally with pneumococcus type II and others with *Streptococcus haemolyticus*. Groups of 5 mice used for the studies on streptococcus were infected intraperitoneally with 200 minimum lethal doses of *Streptococcus haemolyticus*, strain C-203, of which the average minimum lethal dose was 0.5 cc. of 1:10,000,000 dilution of broth culture. Treatment with sulfanilamide or sulfapyridine was given by mouth in a dose of 10 mg. immediately after infection, and once daily for the following two days. Ten milligrams of *p*-aminobenzoic acid or other compound was administered by mouth one hour after each treatment.

* From the Dermatological Research Laboratories, Philadelphia, Pa., Division of Abbott Laboratories, North Chicago, Ill.

[1] While we were engaged in this research, McCarty (9) reported that *p*-aminobenzoic acid is capable of destroying the curative effect of sulfapyridine for type I pneumococcic infection in mice.

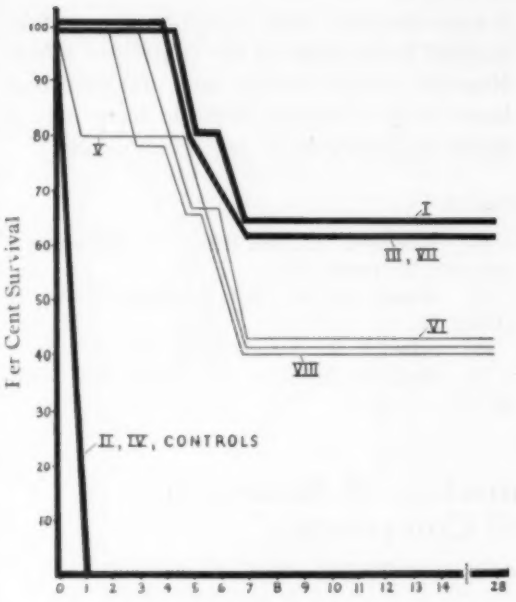

Fig. 1.—Inhibiting Effect of Various Chemical Compounds on Sulfanilamide and Sulfapyridine in *Streptococcus hæmolyticus* Infection in Mice.[1]

(I) Sulfanilamide, (II) sulfanilamide + *p*-aminobenzoic acid, (III) sulfapyridine, (IV) sulfapyridine + *p*-aminobenzoic acid, (V) sulfapyridine + *o*-aminobenzoic acid, (VI) sulfapyridine + *p*-aminobenzenesulfonic acid, (VII) sulfapyridine + thiamin chloride and (VIII) sulfapyridine + *p*-hydroxybenzoic acid.

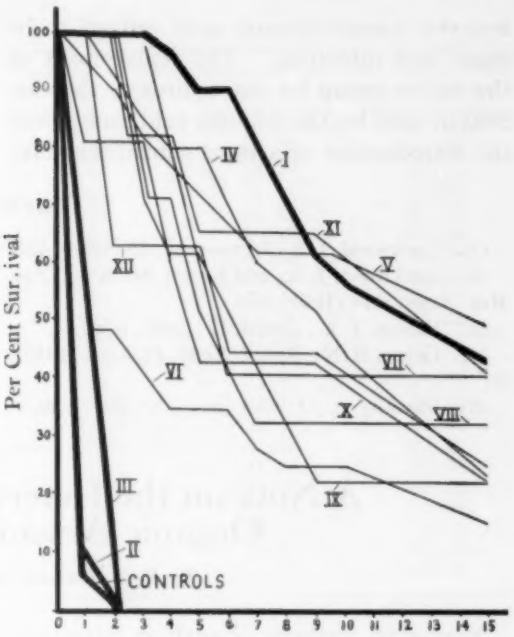

Fig. 2.—Inhibiting Effect of Various Chemical Compounds on Sulfapyridine in Type II Pneumococci Infection in Mice.

(I) Sulfapyridine, (II) sulfapyridine + *p*-aminobenzoic acid, (III) sulfapyridine + *p*-aminobenzamide, (IV) sulfapyridine + *p*-hydroxybenzoic acid, (V) sulfapyridine + *p*-aminobenzenesulfonic acid, (VI) sulfapyridine + *o*-aminobenzoic acid, (VII) sulfapyridine + glycocoll,[2] (VIII) sulfapyridine + 5-acetylaminosalicylic acid, (IX) sulfapyridine + 5-aminosalicylic acid, (X) sulfapyridine + nicotinic acid, (XI) sulfapyridine + thiamin chloride and (XII) sulfapyridine + cevitamic acid.

We found that 100% of streptococcus-infected mice treated with sulfanilamide alone survived for four days and 60% for twenty-eight days, while all mice treated with sulfanilamide plus *p*-aminobenzoic acid died twenty-four hours after treatment. The same was true with sulfapyridine and *p*-aminobenzoic acid. However, other compounds given simultaneously with sulfapyridine seemed to have had slight inhibiting effect.

For the pneumococcic tests, mice were infected intraperitoneally with 10 minimum lethal doses of type II pneumococcus of which the average minimum lethal dose was 0.5 cc. of 1 : 10,000,000 dilution of broth culture. These mice received sulfapyridine orally in doses of 10 mg. three times daily at 9 a. m., 5 p. m. and 12 m. for five days, at 9 a. m. and 5 p. m. on the sixth day, and at 9 a. m. on the seventh day—a maximum of eighteen treatments. Ten milligrams of *p*-aminobenzoic acid or other compounds was given by mouth one hour after each treatment with sulfapyridine.

The *p*-aminobenzoic acid completely inhibited the therapeutic effect of sulfapyridine; all of the 20 animals died after two days. Five mice which were given sulfapyridine plus *p*-aminobenzamide died also after two days—the latter drug probably hydrolyzes in the animal body into *p*-aminobenzoic acid. *p*-Hydroxybenzoic acid (25 mice) and *p*-aminobenzenesulfonic acid (25 mice) did not substantially interfere with the curative effect. *o*-Aminobenzoic acid (15 mice) has partially inhibited sulfapyridine. Glycocoll interferes slightly with the therapeutic effect. 5-Amino- and 5-acetylaminosalicylic acid (10 mice each) showed only a moderate inhibiting action. While nicotinic acid, also cevitamic acid, showed some inhibition, thiamin chloride did not interfere to any appreciable degree with the effect of sulfapyridine, all tested in groups of 5 mice.

[1] Ten milligrams of sulfanilamide or sulfapyridine was given orally immediately after infection, and once daily for the following two days. Ten milligrams of *p*-aminobenzoic acid or other compound was given orally one hour after each treatment.

[2] Glycocoll = aminoacetic acid.

SUMMARY

The therapeutic effect of sulfapyridine by mouth on mice infected with pneumococcus type II or *Streptococcus hæmolyticus* was completely annihilated when *p*-aminobenzoic acid was administered simultaneously. The

isomeric *o*-aminobenzoic acid proved to be much less inhibiting. The replacement of the amino group by the hydroxyl, the carboxylic acid by the sulfonic acid group, and the introduction of a third substituent into *p*-aminobenzoic acid resulted in a substantial destruction of the inhibitory effect. Nicotinic acid, cevitamic acid and even to a lesser degree thiamin chloride have only a slight inhibitory effect on sulfapyridine.

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A Note on the Determination of Arsenic in Organic Arsenical Compounds*

By F. B. Rodman† and Harold N. Wright‡

Numerous methods of analysis have been advocated for the determination of the arsenic content of organic arsenical compounds. Foremost among these are the gravimetric method of Treadwell and Hall (1) and the volumetric method of Lehmann (2). The Treadwell–Hall gravimetric method has been recognized as having a comparatively high degree of accuracy, but is laborious and time consuming. The Lehmann volumetric method was adopted as the official method of assay for arsphenamine and neoarsphenamine in U. S. P. X (1926) and has remained the official method of assay since that time. While the Lehmann method has been considered satisfactory for the assay of arsphenamine and neoarsphenamine it has not been considered satisfactory for the analysis of some of the more refractory arsenicals such as arsanilic acid and sodium cacodylate.

Myers and DuMez (3) made a comparative study of several methods of analysis for the assay of arsphenamine and neoarsphenamine. They assayed a number of specimens of these drugs by four methods, namely (*a*) the Treadwell–Hall gravimetric method, (*b*) Lehmann's, (*c*) Ewins' (4), and (*d*) Gaebel's (5) titration methods. These workers concluded that the Treadwell–Hall and Lehmann methods gave nearly identical results, while the results obtained by Ewins' or Gaebel's methods were invariably low.

Wright, *et al.* (6), in a study of the crystalloid and colloid fractions of arsphenamine and neoarsphenamine, found it impossible to employ the official Lehmann volumetric method in this work since "the oxidation with potassium permanganate and sulfuric acid employed in this method is apparently inadequate to cause the complete breakdown of 'aged' solutions of the arsphenamines, which become decidely refractory to oxidation and almost invariably give low results with the Lehmann method." This was found to be particularly true with the colloidal fractions. Robertson (7) also has concluded that the Lehmann method, while useful in routine assays, is not satisfactory for research purposes.

EXPERIMENTAL

In connection with an investigation of the comparative distribution and retention of the crystalloid and colloid fractions of the arsphenamines to be reported elsewhere (8) a series of comparative analyses of the arsenic content of the sample of neoarsphenamine employed in this study was made by both the Treadwell–Hall and Lehmann methods.

* Presented as a portion of a thesis in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Minnesota.

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TABLE I.—RESULTS OF COMPARATIVE ANALYSIS BY THE TREADWELL–HALL AND LEHMANN METHODS OF THE PER CENT OF ARSENIC IN A SINGLE SAMPLE OF NEOARSPHENAMINE

Analysis No.	Treadwell–Hall Method, Per Cent	Lehmann Method, Per Cent
1	20.17	19.59
2	20.34	19.20
3	20.19	19.27
4	19.92	19.72
5	20.44	19.54
6	20.14	19.55
7	20.24	19.32
8	20.23	19.87
9	20.60	19.82
10	19.81	19.21
11	20.17	19.55
12	...	19.61
13	...	19.79
14	...	19.91
Mean	20.20 ± 0.04	19.57 ± 0.04

This furnished an opportunity to present a statistical analysis of the results of the assay by the two methods. Each analysis represents a different ampul from a batch of drug of a single lot number. All analyses were made in duplicate and the results reported are the means of those duplicate analyses. The results obtained are shown in Table I.

We find therefore that the mean per cent of arsenic found for 11 duplicate analyses from 11 different ampuls of a single lot of neoarsphenamine by the Treadwell–Hall gravimetric method was 20.20% with a probable error of the mean of ±0.04%, while 14 duplicate analyses made similarly by the Lehmann volumetric method gave an arsenic content of 19.57 ± 0.04%.

Applying the familiar formula for the test of a significant difference between means, namely,

$$\text{Sig. Diff.} > 3\sqrt{\text{P.E.M.}_1^2 + \text{P.E.M.}_2^2}$$

we find that a difference between the means in excess of

$$3\sqrt{0.04^2 + 0.04^2} = 0.17$$

would be significant. Since the actual difference between the means is 0.63, this difference is unquestionably significant.

Further evidence of the significance of this difference in the results by the two methods is obtained by a statistical analysis of the results of analyses by Myers and DuMez (3) on 21 samples of arsphenamine of different lot numbers and from several manufacturers. These results are given in Table II.

It may be noted that of these 21 analyses, 18 gave lower results by the Lehmann than by the Treadwell–Hall method, the mean difference being 0.146%, with a probable error of the mean difference of ±0.036. Since the mean difference is well in excess of three times the probable error we may conclude that the Lehmann method also gives significantly lower results than does the Treadwell–Hall method in the assay of arsphenamine.

TABLE II.—ARSENIC CONTENT OF COMMERCIAL SAMPLES OF ARSPHENAMINE (MYERS AND DUMEZ)

Lot No.	Treadwell–Hall Method, Per Cent	Lehmann Method, Per Cent
740	31.58	31.32
750	31.16	31.34
555	31.13	30.94
799	31.52	31.40
809	30.87	30.46
841	31.54	31.38
873	31.38	31.22
886	31.46	31.18
890	31.35	31.22
900	31.07	30.94
928	31.17	31.03
952	31.07	31.03
DBB	31.16	31.22
DFB	31.47	31.32
FBB	31.85	31.50
HBB	31.65	31.28
LBB	31.24	31.20
175	31.15	30.68
176	31.15	31.55
177	31.80	31.61
179	31.56	31.47

The experience of Wright, *et al.* (6), in attempts to employ the Lehmann method in the analysis of dialyzed samples of arsphenamine and neoarsphenamine, would suggest that the low results by the Lehmann method are due to incomplete oxidation of the organic arsenical, particularly of the colloidal component. The probability that the colloid fraction of neoarsphenamine and arsphenamine is resistant to oxidation is also shown by the fact that Wright and Rodman (8) experienced difficulty in the analysis of the colloidal portions of both of these compounds obtained by dialysis when analyzed by the regular Treadwell–Hall method. The use of fuming nitric acid in amounts considerably larger than those recommended by Robertson (7) was required to complete the breakdown of this fraction of the two arsenicals. Since the amount of the colloidal component in these compounds differs not only from one brand to another, but even from one lot to another within the same brand, the degree to which the Lehmann method will give lower results might be expected to vary in the same manner.

SUMMARY

1. The Lehmann volumetric method of analysis for arsenic in organic arsenicals, the official method of assay of the arsenic content of arsphenamine and neoarsphenamine, has been shown to give significantly lower values with both of these drugs than does the Treadwell–Hall gravimetric method.

2. It is suggested that the lower results by the Lehmann method may be due to incomplete oxidation of the organic arsenical, particularly of the colloidal fraction.

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The Synthesis of 3-Hydroxyphthalic Acid

*By Ole Gisvold**

Oxidative studies carried out upon the pigment celastrol, a naturally occurring β-naphthoquinone, yielded a small quantity of material that melted at 244° C. and gave a positive phthalein test. Because the hydroxyl group that is present in this pigment was suspected of being adjacent to the carbonyl groups, 3-hydroxyphthalic acid was an anticipated oxidation fragment. A search of the literature revealed an apparent discrepancy in the melting point of 3-hydroxyphthalic acid. The "Handbook of Chemistry and Physics" gives the melting point of 3-hydroxyphthalic acid as 244° C., and solubilities which corresponded quite closely with those of the above-mentioned oxidation fragment. Heilbron's "Dictionary of Organic Compounds" reports the melting point of 3-hydroxyphthalic acid as 151° C. (with anhydride formation at 161–163° C.) and the melting point of the corresponding anhydride as 198–199° C., with solubilities which differed from those given in the "Handbook." In addition it reports a red color with ferric chloride. No red color with ferric chloride is given by the unknown.

It was therefore necessary to synthesize 3-hydroxyphthalic acid in order to make comparisons with the unknown. No attempt was made to develop good yields of any of the products obtained in this synthesis. 3-Aminophthalic acid was prepared in a pure state in order to report for the first time its melting point. It was not, however, used pure as an intermediate. The reduction of 3-nitrophthalic acid was accomplished catalytically with either platinum or Raney nickel; whereas in the previous syntheses reported in the literature this was accomplished by tin or iron and hydrochloric acid, and no 3-aminophthalic acid was isolated as the intermediate. The 3-hydroxyphthalic anhydride prepared corresponded with that recorded in Heilbron's "Dictionary of Organic Compounds" with respect to solubilities and color reaction. The melting point was somewhat lower; however, no correction was made for stem emergence.

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EXPERIMENTAL

3-Nitrophthalic Acid.—This compound was prepared according to the method given in "Organic Syntheses," Vol. I.

3-Aminophthalic Acid.—The 3-nitrophthalic acid was reduced in alcohol by means of platinum black or Raney nickel at 40 to 50 lbs. pressure and at room temperature. The reduction was accompanied by the formation of large quantities of an unidentified material that appeared to be a polymer. This side reaction was greatest with platinum. The catalyst was removed by filtration, the filtrate concentrated by spontaneous evaporation and the residue digested with water. The aqueous solution was separated from an oily water-insoluble residue. Concentration by spontaneous evaporation yielded crude 3-aminophthalic acid which after several crystallizations from water yielded orange rosettes that melted at 231–232° C. (uncorr.).

3-Hydroxyphthalic Anhydride.—3-Aminophthalic acid was diazotized by the usual method and the crude diazotized product was collected and washed several times with cold water. The diazonium product thus prepared was decomposed according to the directions given in "Organic Syntheses" for the preparation of *m*-nitrophenol. The reaction mixture was then extracted with ether in order to collect the crude 3-hydroxyphthalic acid. Attempts to purify the product by recrystallization from suitable solvents were unsuccessful. The crude material was

then subjected to vacuum sublimation. The sublimate was crystallized from anhydrous ether until a constant melting point was obtained at 195° C. (uncorr.). The 3-hydroxyphthalic acid anhydride thus obtained was very pale yellow in color and gave a deep purple-red color with ferric chloride. It was soluble in water or alcohol.

3-Hydroxyphthalic Acid.—Hydrolysis of a sample of the 3-hydroxyphthalic acid anhydride yielded upon crystallization from anhydrous ether and petroleum ether very pale yellow crystals of 3-hydroxyphthalic acid that melted at 154° C. (uncorr.). This compound was soluble in water or alcohol and gave a deep purple-red color with ferric chloride.

The Influence of Carbohydrates and Salines upon the Separation of Chloral Alcoholate*

By Roland T. Lakey and Carl C. Pfeiffer

The first to record chloral alcoholate incompatibility was G. F. H. Markoe (1), who on June 4, 1885, read a paper entitled "On the Incompatibility of Chloral Hydrate in the Presence of Potassium Bromide and Alcohol" before the Massachusetts State Pharmaceutical Association. He stated that the following prescription was sent to him to determine whether the pharmacist had made a mistake in compounding, because it had subsequently separated into two layers:

℞

Potassium Bromide	
Chloral Hydrate, *āā*	ʒiij
Tr. Opii et Camp.	
Syr. Zingiber, *āā*	ʒiss

After experimenting with this prescription, he came to the conclusion that chloral alcoholate was formed and separated as a top layer. He also observed that potassium bromide, sodium bromide, sodium chloride and magnesium sulfate aided in causing the separation. From his studies he also claimed that calcium bromide, ammonium chloride, ammonium bromide and potassium nitrate did not produce any separation.

G. W. Hargreaves (2) published a study of this type of incompatibility in the June, 1932, issue of the JOURNAL of the AMERICAN PHARMACEUTICAL ASSOCIATION. By analysis, Hargreaves found the oily layer to consist of alcohol, chloral, chloral alcoholate and a small amount of saline. He also found that sucrose in the presence of potassium bromide would cause the separation of chloral alcoholate. These two pertinent papers seem to be the only ones in the English literature on this subject.

This study was prompted by the renewed popularity of chloral hydrate in prescriptions and also by the fact that it has not always been easy to demonstrate this incompatibility to our classes. The purpose, then, of this investigation was to determine the "critical concentration" (a term used by Hargreaves) of each of the ingredients necessary to cause the separation of chloral alcoholate in a chosen basic formula containing chloral hydrate, alcohol, potassium bromide and sucrose.

EXPERIMENTAL

The basic formula chosen for this study is the same as the Elixir of Chloral and Potassium Bromide, Compound, N. F. VI (3):

℞

Chloral Hydrate	12.5 Gm.
Potassium Bromide	12.5 Gm.
Sucrose	10.5 Gm.
Alcohol	...
Water, *q. s. ad.*	50 cc.

One drop of a 1% solution of the fat-soluble dye Sudan III was added to each 50 cc. of the prescription. This serves to delineate any separated chloral alcoholate, since the red-colored dye concentrates in this layer. Kodachrome pictures were taken at the critical point in each experiment. The surface tensions of some of the separated and non-separated solutions were determined by the Du Noüy apparatus to ascertain whether marked changes in surface tension were involved in the separation of chloral alcoholate. All experiments were conducted at room temperature and no heat was used to effect solution of any of the solutes. All of the critical experiments were checked by repetitition. The results of alterations made in the basic formula are given in tabular form.

From Table I it will be observed that separation into two layers occurred with 1% less alcohol when the chloral hydrate was first dissolved in alcohol. This

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Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

is shown in the table by comparing experiment 3 and 4 with experiments 8 and 9.

TABLE I.—ALCOHOL SERIES

PART 1. ALCOHOL ADDED LAST		
Expt. No.	Alcohol, Cc.	Results
1	15	Separates
2	10	Separates
3	6	No separation
4	5	No separation

PART 2. CHLORAL FIRST DISSOLVED IN ALCOHOL		
Expt. No.	Alcohol, Cc.	Results
5	15	Separates
6	7.5	Separates
7	6	Separates
8	5.5	Separates
9	5	No separation

For the rest of the experiments, the formula was made up of 5 cc. of alcohol, and the other ingredients were systematically varied.

TABLE II.—SUCROSE SERIES (CHLORAL HYDRATE FIRST DISSOLVED IN 5 CC. OF ALCOHOL

Expt. No.	Sucrose, Gm.	Results
1	21	Separates
2	15.75	Separates
3	13.13	Separates
4	12.5	Separates
5	11	Separates
6	10.75	Slight separation
7	10.5	No separation

The data of Table II reveal that it requires only a very slight change of sucrose concentration in the base formula to cause separation to take place—1%, *w/v*. This is shown by experiments 5 and 6.

TABLE III.—POTASSIUM BROMIDE SERIES

Expt. No.	Potassium Bromide, Gm.	Results
1	15.75	Separates
2	13.75	Separates
3	13.00	Separates
4 (Check)	13.00	Separates
5	12.6 ($1^1/_{125} \times R$)	Separates

The data of Table III indicate that separations occur even when there is as little as 0.2% increase in potassium bromide over the base formula.

TABLE IV.—CHLORAL HYDRATE SERIES

Expt. No.	Chloral Hydrate, Gm.	Results
1	13	Separates first, then dissolves
2	12.6	Separates first, then dissolves
3	14	Separates first, then dissolves until only a film remains; finally dissolves
4	14	Separates, layer top and bottom; finally rises to top

By changing the concentration of chloral hydrate (Table IV), we find that it requires an addition of only 0.2% of chloral hydrate beyond that of the base formula to produce separation. This is shown by a comparison of experiment 4 with the base formula.

TABLE V

Expt. No.	Potassium Bromide, %, *w/v*	Sucrose, %, *w/v*	Results
1	23	...	Separates
2	22.5	...	Separates
3	...	...	No separation
4	...	56.26	No separation

The data of Table V show that the potassium bromide causes a separation in amounts less than that of the base formula when there is no sucrose present. In this case the chloral alcoholate sinks to the bottom of the bottle. Experiment 3, with no sucrose and no potassium bromide, shows no separation. Experiment 4 shows that even an increase of 56.2%, *w/v*, of sucrose with no potassium bromide present fails to cause a separation. No more sucrose could be dissolved even with several hours of constant agitation and long standing.

Surface tension determinations by the Du Noüy apparatus at 25° C. gave the following: 64.8 dynes/sq. cm. for distilled water, and 44.9 dynes/sq. cm. for the unseparated formula. When separation was effected by the addition of 1% more of alcohol (increased from 11% to 12%), the surface tensions of the layers were, respectively, as follows: chloral alcoholate, 45.3 dynes/sq. cm.; and the water layer, 47.2 dynes/sq. cm.

DISCUSSION

Because of the inability to present the data graphically, it is difficult to visualize the separation of this five- or six-phase system composed of sugar, KBr, water, alcohol, chloral hydrate and chloral alcoholate. That surface tension and interfacial tensions play an important role in the separation of chloral alcoholate cannot be doubted, but here again it is extremely difficult to obtain valid data because altering one constituent such as alcohol is apt to alter the surface tension. Once the separation has occurred, however, a definite change in the surface tension of the two layers has occurred.

The specific gravity of the vehicle also has a role, in that when no sugar is present the chloral alcoholate sinks to the bottom, but when the specific gravity is raised by the addition of sugar the chloral alcoholate then floats to the top. This represents the only danger of this incompatibility, in that the patient might pour the top layer into a glass and obtain all of the chloral at one dose. The work of Adams (7) showing that chloral alcoholate is no more toxic than chloral hydrate has been confirmed in this laboratory by intraperitoneal toxicity studies on the rat.

SUMMARY

Chloral alcoholate will separate from the N. F. prescription when alcohol is added in excess of 10%. In an alcoholic prescription, increasing the sugar beyond 21%, or the KBr beyond 25%, or the chloral hydrate beyond 29% will result in separation. In the absence of sugar the alcoholate will separate when 23% of KBr is dissolved. In the absence of both sugar and KBr, no separation occurs. In the absence of KBr, sugar alone, even if added to the point of saturation, will not cause separation of the alcoholate. The addition of an alcohol-soluble dye to the prescription promptly delineates any separation.

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A Fast Method of Dry, Low-Heat Sterilization*

By P. Goedrich and W. Schmidl

Sterilization, as we all know, is destruction of every form of life, harmful or innocuous. Bacteriologists now agree that any distinction between disease-producing and other microörganisms is vague, and that apparently there is no organism which might not cause disease (1). Even the ordinarily harmless *Bacillus subtilis* (hay bacillus) has been known to cause serious infection in the human eye (2) and occasionally to invade the blood stream (3). It is therefore essential in sterilization to destroy not only all vegetative forms of bacilli, but also all spores. Unfortunately, there are wide discrepancies in the results reported concerning the thermal resistance of spores. But all authorities agree that no period of exposure to boiling water has been found to be completely adequate. In the opinion of leading bacteriologists, boiling water never constitutes adequate sterilization for surgical instruments (4).

Robert Koch (5), who established that all vegetative forms of bacteria were killed by a temperature just over 100° C. in 1½ hrs., overestimated the value of boiling water against spores. Falcioni (6) found spores of tetanus to resist destruction for 2½ hrs. in live steam. Theobald Smith (7) showed that tetanus spores occasionally were able to survive 70 min. of steaming. Von Hibler (8) studied some strains of *Clostridium tetani* that required 3 hrs. of boiling to destroy them. Bigelow and Esty (9) found some microörganisms remaining virulent for 22 hrs. at 100° C., while at 110° C. nearly 4 hrs. was necessary to destroy them. Becker (10) reported the death point of two strains of tetanus as 2 hrs. and 3 hrs., respectively, in boiling water. Murray and Headler (11) found strains of *Clostr. welchii* to be resistant to boiling for 90 min. Esty and Meyer (12) found the heat resistance of tetanus spores to vary at 100° C. from 15 to 90 min., the average survival time being 25 min. Dried spores of *B. anthracis* were found to withstand boiling temperature for hours (13).

In general it is recognized that a temperature of 150° C. continued for at least an hour will destroy any bacterial spores. Nevertheless, glassware in the bacteriological laboratory is usually sterilized at 160° C. for 2 to 3 hrs. (14). By using steam

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Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

under pressure, 30 to 40 min., with a pressure of 15 lbs. (121.5° C. at sea level), provide a satisfactory margin of safety (15). In order to employ steam without pressure, one submits the material to intermittent heating on three consecutive days, using apparatus of the usual "steam sterilizer" type. But according to Theobald Smith (16, 17) even this time-consuming procedure is not reliable. Abbott (18) also states that, in general, dry objects like instruments, bandages, etc., do not offer the conditions for the operation of the principles underlying the intermittent method of sterilization. It must also be considered that the boiling point of water is lower at higher altitudes, but the death of the microörganisms depends on the temperature and not on the boiling point. Thus steam sterilization, already doubtful at sea level, becomes worthless at mountainous elevations.

From the foregoing it appears that there is need for a new inexpensive way of sterilization for instruments, syringes, hypodermic needles, etc., in physicians' and dentists' offices and everywhere that a complete sterilization is wanted without the use of autoclaves or dry heat at 160° C.

As early as 1902, Esmarck (19) had shown that the action of live steam is markedly increased by addition of small quantities of volatile chemical disinfectants like formaldehyde, chlorine, etc. Spores of potato bacillus which were killed in live steam at 100° C. in 2 hrs., were killed in 2 min. by steam to which 1% of formaldehyde was added. Clemmensen (20) found that materials would be sterilized at a temperature from 68° to 70° C. when exposed to formaldehyde–water vapors. Likewise Delepine found that anthrax spores may be easily killed by exposure to warm formaldehyde vapors (21). But this kind of sterilization was qualified as "possible but not practical" (22), the apparatus necessary being even more complicated and more expensive than autoclaves.

The new method, reported in this paper, uses the vapors of certain dry germicides in closed containers and effects complete sterilization in a relatively short time and at low heat. For practical reasons paraformaldehyde (trioxymethylene) appears to be the most promising one among many theoretically possible substances, such as thymol, chlorthymol, chloramine, etc.

EXPERIMENTAL

The method consists of evaporation of paraformaldehyde by comparatively moderate heat in a closed chamber. The materials to be sterilized are contained in individual receptacles contained in this master-chamber. These receptacles can be closed by revolving shutters or by other means after the sterilization period has elapsed. After the receptacles are taken out, the instruments, etc., contained in them, will stay sterile for a prolonged time.

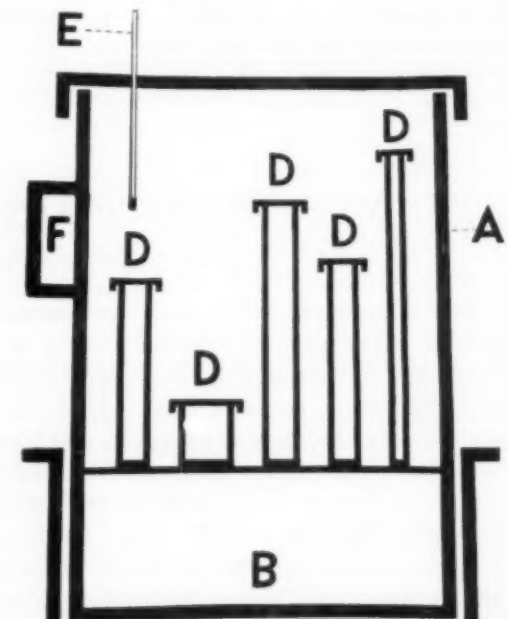

Figure 1.

Figure 1 illustrates the principles of the new method. In the master-chamber (*A*), hermetically closed, paraformaldehyde spread over the bottom (*B*) is evaporated by means of the heating element (*C*). This master-chamber encloses several individual receptacles (*D*) containing the instruments and the other articles to be sterilized. The receptacles are provided with revolving shutters, the shutters being opened before sterilization. A thermometer (*E*) registers the temperature. After the sterilization time has elapsed, the master-chamber may be taken away with the handle (*F*), cooled and opened. The revolving shutter of each individual container is closed and the instruments, etc., remain in it in sterile condition until they are wanted for use. It has been found, moreover, that the disinfectant vapors penetrate even closed glassine paper envelopes, and

thus make their opening before sterilization and closing thereafter unnecessary.

The experiments conducted with paraformaldehyde demonstrated that the liberation of formaldehyde depends on (*a*) the temperature, (*b*) the time element and (*c*) the exposed surface. Table I demonstrates the liberation of formaldehyde, at a constant temperature in closed containers of constant capacity, from various amounts of paraformaldehyde.

TABLE I.—EVAPORATION OF VARIOUS QUANTITIES OF PARAFORMALDEHYDE IN 1000-CC. CLOSED CONTAINERS AT 70° C.

Quantity, Gm.	Loss of Weight after One Hour	
	Gm.	Per Cent
0.014	0.0038	27
0.05	0.0155	31
0.5	0.091	18
1.0	0.139	14
2.0	0.180	9

Table II shows the liberation of formaldehyde in closed containers of various capacities from various quantities of paraformaldehyde at a constant temperature.

The most important question was, of course, the investigation of the sterilizing effect of this method. Formaldehyde is known to have one of the greatest temperature coefficients of any disinfectant (23). Holm and Gardener (24), investigating formaldehyde sterilization, recognized that it is the coöperation of four elements, (*a*) concentration of the fumigant, (*b*) temperature, (*c*) time of exposure and (*d*) moisture, that determines sterilization.

Realizing that very small amounts of moisture also improve the killing action of heat (25), it is possible to comply with the requirement of moisture and nevertheless have the material virtually dry, as 1000 cc. of steam at 100° C. under atmospheric pressure has a weight of only 0.6 Gm. It is obvious that a few drops of water per L. volume may suffice without injuring even sensitive objects. The investigation was conducted at various temperatures, at various times of exposure and at various concentrations of the vapors of paraformaldehyde.

Table III shows the results of the new method, when using an F. D. A. culture of *Staphylococcus aureus*.

It can be seen on this table that, while it was impossible to achieve sterilization at 20° C. in three days, the bacilli were killed in 45 min. at 70° C., and in 10 min. at 90° C. Dry heat of 100° C. did not kill the same strain of bacteria in 1 hr. If one accepts the very much disputed theory that there is generally little likelihood that instruments may be infected with organisms of the very resistant type, this short-time "sterilization" might suffice.

Ten minutes at 90° C. with the new method produces at least the same results as short-time boiling or steaming in the usual office sterilizers, and whoever has to rely on this kind of sterilization may use short-time exposure to the vapors of paraformaldehyde and enjoy the advantage of having dry instruments, in individual containers, ready for immediate or later use. But for higher requirements, for instance, the destroying of all spores, the new method assures a complete sterilization, which boiling or steaming at 100° C. never can; by simply prolonging the time of exposure to the vapors of paraformaldehyde this is effected.

In order to determine this effectiveness against spore-bearing organisms, instruments were artificially infected with such organisms, previous to the sterilization. Saline suspensions of strains of *B. subtilis*, which were grown on slants of F. D. A. solid medium and which had survived more than 2½ hrs.' exposure to 7% U. S. P. Tincture of Iodine, were used (½-cc. saline suspensions in 10 cc. of tincture). The saline suspensions were made of 10 cc. of saline solution per one 10-cc. slant of F. D. A. solid medium. The same strains were found to be resistant to dry heat for 1½ hrs. at

TABLE II.—EVAPORATION OF VARIOUS QUANTITIES OF PARAFORMALDEHYDE IN CLOSED CONTAINERS OF VARYING CAPACITY AT 70° C.

Quantity, Gm.	Loss of Weight in Containers of							
	500-Cc. Capacity		1000-Cc. Capacity		2500-Cc. Capacity		5000-Cc. Capacity	
	Gm.	Per Cent	Gm.	Per Cent	Gm.	Per cent	Gm.	Per Cent
0.1	0.017	17	0.028	28	0.04	40	0.05	50
0.5	0.05	10	0.091	18	0.11	22	0.14	28
1.0	0.057	5.7	0.139	14	0.15	15	0.20	20

TABLE III.—KILLING OF BACTERIA WITH VAPORS OF PARAFORMALDEHYDE (TEST ORGANISM, *Staph. Aureus*, F. D. A. CULTURE)

Temperature, °C.	Amount of Paraformaldehyde per 1000 Cc.	Time of Exposure									
		10 Min.		20 Min.		30 Min.		45 Min.		3 Days	
		Broth Culture	Subculture	Broth Culture	Subculture	Broth Culture	Subculture	Broth Culture	Subculture	Broth Culture	Subculture
20	Excess	..	..	..	..	..	..	..	..	−	+
70	0.6 Gm.	..	..	..	..	−	+	−	−	..	..
80	0.6 Gm.	..	..	+	+	−	−	..	..	..	..
90	0.6 Gm.	−	−	..	..	..	..	..	..	..	..

TABLE IV.—KILLING OF SPORES WITH VAPORS OF PARAFORMALDEHYDE (TEST ORGANISM, *Bacillus subtilis*)

Temperature, °C.	Amount of Paraformaldehyde per 1000 Cc.	Time of Exposure: 45 Min. Broth Culture	45 Min. Subculture	60 Min. Broth Culture	60 Min. Subculture	90 Min. Broth Culture	90 Min. Subculture	1 Day Broth Culture	1 Day Subculture	3 Days Broth Culture	3 Days Subculture	8 Days Broth Culture	8 Days Subculture
20	Large excess	..	..	..	..	..	..	+	+	+	+	+	+
37	Large excess	..	..	..	..	..	..	+	+	−	−	..	..
60	1 Gm.	..	..	+	+	+	+	..	..	..	..	..	..
70	1 Gm.	..	..	+	+	+	+	..	..	..	..	..	..
80	0.4 Gm.	..	..	+	+	+	+	..	..	..	..	..	..
80	0.6 Gm.	..	..	−	+	−	+	..	..	..	..	..	..
90	0.2 Gm.	+	+	+	+	..	..	..	..	..	..	..	..
90	0.4 Gm.	+	+	−	+	..	..	..	..	..	..	..	..
90	0.6 Gm.	−	+	−	−	..	..	..	..	..	..	..	..

130° C., and to live steam under atmospheric pressure for one hour at least. A wide variety of objects, such as glass tubes, glass rods, strings, wood, nails, needles, metal instruments, empty ampuls, etc., were infected and dried after infection at 37° C., then put into individual containers and exposed to the vapors in the closed chamber. To ascertain the resistance of the strains of *B. subtilis*, appropriate controls without formaldehyde were always conducted.

As Table IV indicates, the spores of *B. subtilis* were not killed even after 8 days of exposure at 20° C, but 3 days at 37° C. and 60 min. at 90° C. were sufficient for complete sterilization.

As it is a well-established fact that organic matter reduces the effect of all germicides, and as it might happen that instruments are not free of blood, serum, pus, etc., before sterilization, two series of experiments were conducted, using (*a*) instruments which were immersed in a mixture of equal amounts of horse serum and saline suspensions of *B. subtilis* and (*b*) instruments which were immersed in horse serum, dried at 37° C., and then infected with *B. subtilis*. Even under these rigid conditions the results were the same as indicated in Table IV—complete sterilization, with destruction of all spores, could be achieved in the same period of time.

It has already been mentioned that the sterilized articles, contained in their individual receptacles, remained sterile, after they had been taken out of the master-chamber, for a prolonged time. When sterility tests were made, after the sterilized articles in their individual containers had been kept without special precautions for one, two and even three months, there was not a single case of subsequent infection.

The heat necessary for the experiments with this new method was provided by a thermostat-controlled electric oven. In practice, suitable means for heating and controlling the predetermined temperature will easily be provided. But as the temperature required for complete sterilization never exceeds 90° C., simple immersion of the closed master-chamber into boiling water for the time indicated (see Table IV) will be sufficient where means of electric or gas heating are not available.

Formaldehyde fumes are known to be irritant to the respiratory tract, but it was found that this can be practically eliminated by letting the master-chamber cool to room temperature, or, even better, by cooling it with tap water. This will polymerize formaldehyde again to the solid form of paraformaldehyde.

Quantitative determination of the deposit of paraformaldehyde on the surface of the sterilized instruments showed that it is in the average 0.00008 Gm./sq. cm. immediately after cooling, and 0.000015 Gm./sq. cm. (less than $^1/_{4000}$ gr.) after 24 hrs., an undoubtedly negligible amount.

SUMMARY

1. Neither boiling water nor live steam constitutes a reliable means of sterilization.

2. The routine "sterilization" of surgical instruments by short-time boiling or exposing to live steam at 100° C. may therefore have dangerous consequences.

3. A new method of sterilization of surgical instruments and other articles has been found, by which the same results are obtained as with a steam pressure autoclave.

4. The method consists of exposing the instruments, etc., to the vapors of formaldehyde, developed in a master-chamber, from paraformaldehyde under virtually dry conditions.

5. The method enables sterilization of instruments, etc., in individual containers, which, being properly closed after finished sterilization, will keep the instruments sterile for a prolonged time and ready for their use at the doctor's office, as well as for emergency calls.

6. Complete sterilization of heat-sensitive articles may be achieved at temperatures as low as 37° C., if the time element is not of major significance. At 90° C. one hour of

exposure is sufficient to kill spores of even extraordinary resistance.

7. The new method requires only small quantities of an inexpensive chemical and can be used in locations and under conditions where the bulky and heavy autoclave cannot be considered. The apparatus needed for this method is very simple, easy and safe to handle, and requires little attendance.

8. This new method may be of special importance for the armed forces, when their mobile hospitals are unable to use autoclave sterilization.

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A Histological Study of *Eriodictyon californicum**

By L. David Hiner and Kenneth J. Merrill†

Criticism of the United States Pharmacopœial description of *Eriodictyon californicum* (*Hydrophyllaceæ*) has led the Committee on Botany and Pharmacognosy to request that a further study of the drug be made. In accordance with this suggestion, an investigation of some of the histological features of the leaves was undertaken by Mr. Merrill during the past year. It was deemed advisable, however, to continue the study this summer, so additional samples were obtained for corroborative work as well as for checking additional points which were still in question. The study was based chiefly on a review of the older descriptions, and also on the newly proposed monograph resulting from the work of Drs. Youngken, Wirth and Goodrich of the present Committee.

Since the first histological studies of this drug which were made by Ritter (1), it appears that very little interest has been shown in the leaves until this Committee expressed its disapproval of the existing monograph. Some definite changes are suggested herewith as a result of their proposal to revive the study of *Eriodictyon californicum*, and ultimately to revise the monograph.

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Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

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HISTOLOGY OF THE LEAF

The following characteristics have been noted in the study of the leaf structure.

Transverse Section.—The upper epidermis is composed of large, clear cells with thick walls, oval or rectangular shaped, with the surface edges wrinkled. Glandular hairs with 1- to 3-celled stalks and multicellular heads occur abundantly on this surface. They are deep seated and have very short stalks, which is in agreement with the findings reported by other investigators (see Fig. 1).

Fig. 1.—Deep-Seated Trichomes and Wrinkled Epidermis.

In a transverse and surface view of this same upper epidermis the cells are polygonal or hexagonal shaped and appear to be deeply striated due to the wrinkled surface of the epidermis. The striations are more prominent around the base of the glandular hairs and therefore appear to be formed by the epidermis pushing against the stalks of the trichomes (see Fig. 2). The tendency to wrinkle was more noticeable in the turgid cells.

Many glandular hairs are present on the upper epidermis, seeming to form at the junction of the epidermal cells rather than to develop from any one particular cell. The heads of these hairs are multicellular, occasionally containing a yellowish brown, granular secretion. Preliminary cell counts of these multicellular heads raised another question, when it was found that they were not always in agreement with the "up to 8-celled heads," as given in the official description of the newly proposed monograph. Therefore it was deemed advisable to make additional studies of this point.

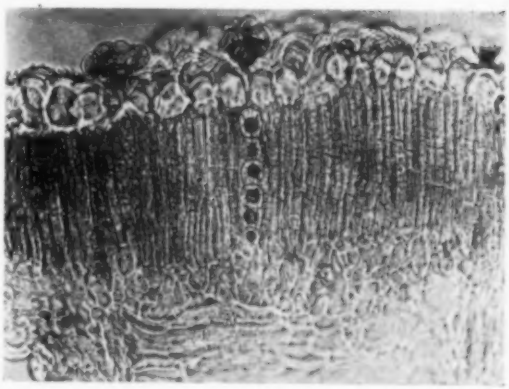

Fig. 2.—Epidermal Striations and Glandular Hairs.

Samples of *Eriodictyon californicum* were obtained from several reliable laboratories and crude drug dealers, and a total of 137 leaves selected at random from 11 commercial samples of the drug were prepared and examined. A Howe ocular counting chamber was used in the procedure, and the investigations were carried out on the surface sections using the oil immersion lenses. Unfortunately it was impossible to record the findings photographically inasmuch as the cells of the glandular heads did not all come into focus at the same level. They were frequently piled on top of each other to a depth of three distinct layers of cells.

Trichomes containing as high as 16-celled heads were discovered, although such an occurrence was rare indeed. A record of a typical count made from equal area surface sections of 11 different Eriodictyon samples is contained in Table I.

It is easily evident that the "up to 8-celled heads" predominate, but it is also noteworthy that trichomes with heads containing cells in excess of that number are present. This is not in agreement with the now proposed monograph, and needs additional study (2).

In preparing the leaves for making these surface section studies, they were macerated in chloral hydrate solution for at least 24 hrs. Prior to cutting the sections, the leaves were washed with fresh chloral hydrate solution to remove the dissolved resinous material. The glandular hairs showed

TABLE I.—CELL COUNT OF GLANDULAR HAIRS

Sample	Number of Head Cells												
	4	5	6	7	8	9	10	11	12	13	14	15	16
M-1	8	5	5	15	36	15	12	2	3	1	0	1	1
S-0	0	2	3	15	40	3	2	0	1	0	1	0	0
F-2	6	3	12	7	26	4	5	2	1	0	1	0	0
S-a	3	2	0	19	22	4	1	1	0	0	0	0	0
L-o	5	10	5	11	31	3	4	0	1	0	0	0	0
L-n	23	12	18	30	50	24	8	4	2	0	0	1	0
P-g	15	0	0	0	23	0	2	1	0	0	0	0	1
O-291	6	5	0	8	15	0	5	1	0	0	0	0	0
W-m	3	5	5	27	85	17	12	1	2	0	0	0	0
W-s	7	6	9	1	24	0	3	0	0	0	0	0	0
I-1	1	2	4	5	17	0	0	0	0	0	0	0	0

well in sections prepared in this way, when they were mounted in the same solution. Washing or mounting the sections in water, alcohol or glycerin alone collapsed the heads so that the number of cells present could not be distinguished.

The palisade layer consists of very narrow cells, 2 to 6 rows deep, and filled with chloroplastids. Viewed from above through the epidermal cells they appeared as masses of very small cells, sometimes as many as 10 appearing within the boundaries of a single epidermal cell. The palisade cells thin out at the edge of the leaf to a single layer of cells which may extend to the lower epidermis.

Merrill found that between the palisade cells at more or less regular intervals there are perpendicular rows of large parenchyma cells bearing rosette crystals of calcium oxalate. These cells are larger than the palisade tissue, have thicker walls, and do not contain chloroplastids. They vary mostly from 4 to 9 cells in the rows, are very conspicuous, and appear to be a very diagnostic feature of *Eriodictyon californicum*. They are best observed when the tissue has been cleared with chloral hydrate (see Fig. 3).

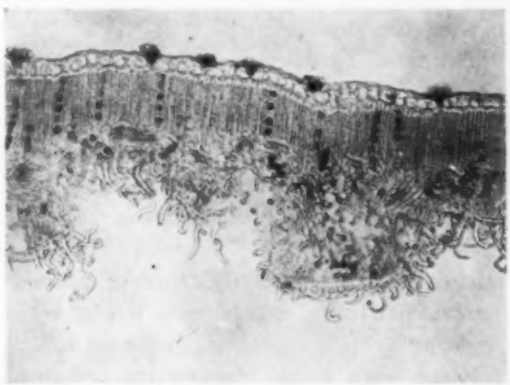

Fig. 3.—Rosette-Bearing Parenchyma.

Cross sections through the midrib of the leaf show the lower epidermis bearing occasional glandular hairs with multicellular heads. The number of cells in these heads vary from 4 to 8, with the 8-celled heads occurring most frequently. No nonglandular hairs were found growing on the lower epidermis of the midrib; however, this contingency must be taken into consideration. Radial longitudinal sections through the midrib may show the epidermis appearing to bear nonglandular hairs if the cut ends adjacent to the surface of the lamina. Nonglandular hairs abound in this sinus, and in the isolated sections may appear to have come from the lower epidermis of the midrib proper (see Fig. 4). No nonglandular hairs were observed on the upper epidermis of the midrib structure. All other histological features of the midrib structure were found to be in complete agreement with the findings as reported by other investigators. Calcium oxalate crystals were present.

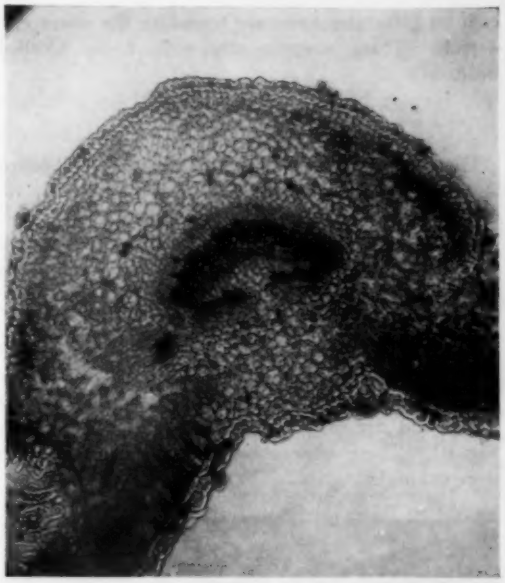

Fig. 4.—Nonglandular Hairs in Lamina-Midrib Sinus.

Spongy parenchyma is conspicuous, increasing in depth around the more prominent veins. The vascular system through the spongy parenchyma is composed chiefly of spiral trachea. Pitted trachea were not numerous, and were found mostly in the older leaves.

The lower epidermis is composed of thin-walled cells and masses of unicellular nonglandular hairs. These hairs are undulate and fill the sinuses formed by the more prominent veins.

Sections showing the stomata reveal them as nearly round, and having an oval-shaped opening between the guard cells (see Fig. 5). Rarely multi-

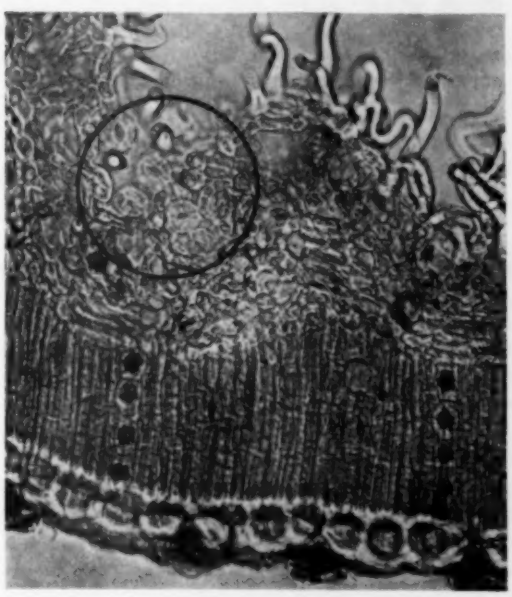

Fig. 5.—Round Stomata.

cellular glandular hairs are found on the lower epidermis. These were mostly with 2- to 4-celled heads.

SUMMARY

Glandular hairs were found to have 1- to 2-celled stalks as reported in the Committee Bulletin; 3-celled stalks also were found.

Glandular hairs containing multicellular heads varying from 4 to 16 cells were found; 9- to 10-celled heads were frequent.

Calcium oxalate rosette aggregates occur perfectly aligned in perpendicular rows of parenchyma cells interspersed in the palisade region. These cells are mostly from 4 to 9 cells deep, have thicker walls, and are larger than the palisade cells (see Fig. 6). These were observed in all leaves which were examined.

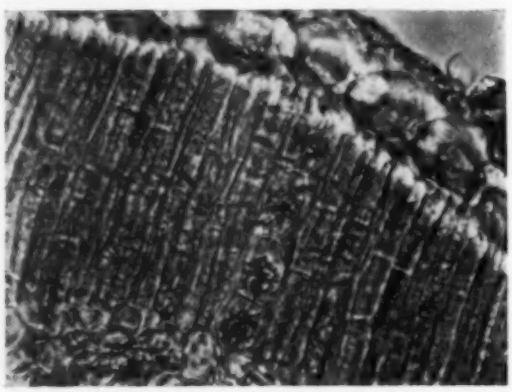

Fig. 6.—Rosettes of Calcium Oxalate.

Calcium oxalate crystals in rosette aggregates extend throughout the length of the midrib. The crystals may be present in two rows of cells that are adjacent to each other, but usually only one row of parenchyma cells holds the aggregates.

CONCLUSIONS

It is recommended that the following changes as indicated be incorporated in the official monograph of Eriodictyon.

Histology: Upper epidermis with large cells, the outer walls very uneven in cross sections, owing to indentations which appear as striations in surface view, and with numerous deep-seated glandular hairs, possessing a 1- to 3-celled stalk *and a multicellular glandular head with up to 8 cells predominating*, but nonglandular hairs absent on this epidermis. Palisade cells very narrow, from 2 to 6 rows deep, *in which are interspersed regularly arranged perpendicular rows of parenchyma cells, nearly each one of which contains a calcium oxalate rosette aggregate.* Cells of spongy parenchyma forming but a few layers; vascular tissues not strongly developed except in the midrib and more prominent veins; lower surface in cross section showing numerous sinuses lined by the lower epidermis from which occasional deep-seated glandular hairs and many curved nonglandular hairs emanate and nearly fill the sinuses, frequently concealing the stomata.

Powdered Eriodictyon: Greenish yellow; nonglandular hairs 1-celled, undulate with thick walls, up to 250 μ in length and up to about 10 μ in width; glandular hairs with 1- to 3-celled stalks *and multicellular heads with up to 8 cells predominating, the latter up to 120 μ in diameter; fragments of palisade tissue containing regularly arranged columnar parenchyma cells, nearly each one of which contains a rosette aggregate of calcium oxalate;* tracheæ with spiral thickenings or simple pores and associated with lignified fibers; calcium oxalate in rosette aggregates numerous and from 5 to 30 μ in diameter.

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Incompatibilities in Prescriptions

V. The Use of Tribasic Calcium Phosphate and Silica Gel in Capsules to Prevent Liquefaction*

By William J. Husa† and Thomas J. Macek‡

In previous papers, Husa and Becker (1) reported on the value of different inert powders in capsules to prevent liquefaction due to deliquescence or the formation of an eutectic mixture. Among the inert powders they used were light magnesium oxide, heavy magnesium oxide, magnesium carbonate, talc, lactose and several different starches, both dried at 100° C. and undried. Since the publication of the above reports, two other substances, tribasic calcium phosphate and silica gel, have been studied similarly. In the present paper a report is made on the value of both of these substances as inert ingredients in capsules that liquefy. A comparison has likewise been made between these powders and light magnesium oxide and magnesium carbonate, which were found in the previous experiments to be most effective in preventing liquefaction of capsules.

The tribasic calcium phosphate was a fine, white powder of the grade specified in the monograph for Tribasic Calcium Phosphate in the Second Supplement to the U. S. P. XI. The silica gel used in the present investigation was a fine powder, very faintly pink in color, and was supplied through the courtesy of the Davison Chemical Company, Baltimore, Maryland. The other inert ingredients, light magnesium oxide and mangesium carbonate, as well as the ingredients of the capsule prescriptions, were of U. S. P. grade.

EXPERIMENTAL

PRESCRIPTION NO. 1

℞	Camphor	gr. 1/4
	Salol	gr. iij

Fac tales capsulæ no. xx.
One capsule t. i. d. a. c.

When the ingredients of this prescription were triturated a pasty mass was formed. The prescription was compounded with varying quantities of the inert powders to determine the relative efficiency of each in preventing the difficulty. In each case the camphor was triturated with the inert powder; the salol was then added and the mixture triturated lightly. The temperature at the time of compounding was 71° F.; the relative humidity was 23–25%. The finished capsules were kept under observation for two weeks in open beakers as well as in closed capsule vials.

The addition of an inert powder obviously increases the size of the capsule to a certain extent depending on the quantity of powder used and its bulkiness. In the present investigation capsules larger than those ordinarily dispensed in prescription practice were used in some cases. This was done for purposes of uniformity in the experimental work. In many cases where the patients find it difficult to swallow the larger capsules it would be advisable to divide the material of the prescription into twice the number of capsules designated by the prescriber and to double the dose as to the number of capsules. The size of the capsule required in each case of the present study was recorded.

In all the tables of the present paper the following abbreviations are used: L = soft mass or liquid; P = dry powder; D = damp powder; S D = slightly damp powder; C M = cement-like mass. The quantity of inert powder is stated in grains per capsule.

Table I shows that magnesium carbonate was most effective in preventing liquefaction, 1 grain per capsule being sufficient to maintain the ingredients as a powder for four days. One grain of light magnesium oxide per capsule maintained the ingredients as a powder for two days. In the case of silica gel and also with calcium phosphate, 1 grain of the powder per capsule was not sufficient to keep the ingredients as a powder even during the period of compounding; 2 grains or more per capsule, however, were sufficient to maintain the ingredients as a powder for two weeks.

Table II shows that the results with the capsules stored in closed vials were similar to those with the capsules placed in open beakers. For this prescription at least 2 grains of one of the inert ingredients is indicated. Tight closure of the container seems to be without any distinct advantage.

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* Presented to the Section on Practical Pharmacy of the A. PH. A., Detroit meeting, 1941.

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‡ Graduate Assistant in Pharmacy, University of Florida, 1938–1940.

TABLE I.—PRESCRIPTION NO. 1 WITH INERT POWDERS

(Capsules Stored in Open Containers)

Inert Powder, Grains	Size of Capsule	Time in Days 0	1	2	4	14
Magnesium Carbonate						
1	2	P	P	P	P	D
2	1	P	P	P	P	S D
3	0	P	P	P	P	P
4	00	P	P	P	P	P
5	000	P	P	P	P	P
Light Magnesium Oxide						
1	2	P	P	P	S D	S D
2	0	P	P	P	P	P
3	00	P	P	P	P	P
4	000	P	P	P	P	P
Silica Gel						
1	3	S D	S D	S D	S D	D
2	2	P	P	P	P	P
3	1	P	P	P	P	P
4	0	P	P	P	P	P
5	00	P	P	P	P	P
Tribasic Calcium Phosphate						
1	3	S D	D	D	D	D
2	2	P	P	P	P	P
3	1	P	P	P	P	P
4	0	P	P	P	P	P
5	0	P	P	P	P	P

TABLE II.—PRESCRIPTION NO. 1 WITH INERT POWDERS

(Capsules Stored in Closed Containers)

Inert Powder, Grains	Size of Capsule	Time in Days 0	1	2	4	14
Magnesium Carbonate						
1	2	P	P	P	P	D
2	1	P	P	P	P	P
3	0	P	P	P	P	P
4	00	P	P	P	P	P
5	000	P	P	P	P	P
Light Magnesium Oxide						
1	2	P	P	P	S D	S D
2	0	P	P	P	P	P
3	00	P	P	P	P	P
4	000	P	P	P	P	P
Silica Gel						
1	3	S D	S D	S D	S D	D
2	2	P	P	P	P	P
3	1	P	P	P	P	P
4	0	P	P	P	P	P
5	00	P	P	P	P	P
Tribasic Calcium Phosphate						
1	3	S D	D	D	D	D
2	2	P	P	P	P	P
3	1	P	P	P	P	P
4	0	P	P	P	P	P
5	0	P	P	P	P	P

PRESCRIPTION NO. 2

℞	Acetylsalicylic acid	gr. iiss
	Methenamine	gr. iiss

Ft. caps. d. t. d. no. xii.

In filling this prescription a damp powder resulted. After one day the contents of the capsules liquefied and on longer standing a light yellow color developed.

Prescription No. 2 was filled with the addition of inert powders. The temperature at the time of compounding was 74° F.; the relative humidity was 24–27%. In each case the methenamine was mixed with the inert powder and the acetylsalicylic acid added, the final mixture being triturated lightly.

Table III shows that light magnesium oxide is the most effective powder in preventing liquefaction of the ingredients of the capsule. The ingredients of the capsule can be maintained as a powder for four days when stored in open containers

TABLE III.—PRESCRIPTION NO. 2 WITH INERT POWDERS

(Capsules Stored in Open Containers)

Inert Powder, Grains	Size of Capsule	Time in Days 0	1	2	4	14
Magnesium Carbonate						
1/2	2	P	C M	C M	L	L
1 1/2	1	P	C M	C M	L	L
2 1/2	0	P	S D	S D	D	L
3 1/2	00	P	P	P	S D	D
Light Magnesium Oxide						
1/2	1	P	C M	C M	C M	L
1 1/2	0	P	P	P	D	L
2 1/2	00	P	P	P	S D	D
3 1/2	000	P	P	P	P	S D
Silica Gel						
1/2	2	P	C M	C M	L	L
1 1/2	1	P	C M	C M	L	L
2 1/2	0	P	C M	C M	L	L
3 1/2	00	P	C M	C M	D	L
Tribasic Calcium Phosphate						
1/2	2	S D	C M	C M	L	L
1 1/2	1	P	C M	C M	L	L
2 1/2	0	P	C M	C M	L	L
3 1/2	00	P	S D	S D	D	L

TABLE IV.—PRESCRIPTION NO. 2 WITH INERT POWDERS

(Capsules Stored in Closed Containers)

Inert Powder, Grains	Size of Capsule	Time in Days 0	1	2	4	14
Magnesium Carbonate						
1/2	2	P	C M	C M	C M	C M
1 1/2	1	P	C M	C M	C M	C M
2 1/2	0	P	P	P	S D	D
3 1/2	00	P	P	P	P	S D
Light Magnesium Oxide						
1/2	1	P	P	C M	C M	C M
1 1/2	0	P	P	P	P	P
2 1/2	00	P	P	P	P	P
3 1/2	000	P	P	P	P	P
Silica Gel						
1/2	2	P	C M	C M	C M	C M
1 1/2	1	P	C M	C M	C M	C M
2 1/2	0	P	C M	C M	D	C M
3 1/2	00	P	C M	C M	D	C M
Tribasic Calcium Phosphate						
1/2	2	S D	C M	C M	C M	C M
1 1/2	1	P	C M	C M	C M	C M
2 1/2	0	P	C M	C M	C M	C M
3 1/2	00	P	S D	S D	C M	C M

if 3½ grains of light magnesium oxide are added per capsule. Magnesium carbonate, silica gel and tribasic calcium phosphate are relatively inefficient as inert powders in this case since the ingredients of the capsules form a cement-like mass or liquefy even when larger quantities of these inert powders are added per capsule.

There is a slight advantage in storing the capsules of prescription No. 2 in closed containers. Table IV indicates that light magnesium oxide is the most effective powder in this case, 1½ grains per capsule being sufficient to maintain the ingredients of the capsule as a powder for fourteen days. Although magnesium carbonate is effective in some cases, silica gel and tribasic calcium phosphate are relatively ineffective.

PRESCRIPTION NO. 3

℞ Methenamine gr. ij
Potassium acetate gr. ij

Prepare 30 such capsules.

When this prescription was compounded as written, a damp powder formed which liquefied in the capsules within a few days. Due to the removal of moisture from the gelatin capsules by the deliquescent potassium acetate, the capsules became brittle and cracked within a few minutes after filling. Storage of the capsules in closed vials maintained the powdered character of the ingredients over a period of two weeks; however, the capsules became brittle and cracked.

The prescription was compounded using various amounts of inert powders. In all cases of compounding, the methenamine was first triturated to obtain a fine powder and then the absorbent and potassium acetate were added in the order named. The temperature during compounding was 73–75° F.; the relative humidity was 21–23%.

Table V indicates that prescription No. 3 has a tendency to become damp or liquid, accompanied by brittleness and cracking of capsules, when stored in open containers, even when inert powders are added. Table VI shows that dampening and liquefaction of the capsules are retarded to a considerable extent when the capsules are stored in closed containers and when inert powders are added. Light magnesium oxide is the best inert powder in this case, followed in order by magnesium carbonate, tribasic calcium phosphate and silica gel.

TABLE V.—PRESCRIPTION NO. 3 WITH INERT POWDERS

(Capsules Stored in Open Containers)

Inert Powder, Grains	Size of Capsule	Time in Days: 0	1	2	4	14
Magnesium Carbonate						
1	2	P[a]	D[a]	L[a]	L[a]	L[a]
2	1	P	S D	S D	D[a]	L[a]
3	00	P	S D	S D	D[a]	L[a]
4	000	P	S D	P	S D	D[a]

(Table V continued in next column)

Inert Powder, Grains	Size of Capsule	Time in Days: 0	1	2	4	14
Light Magnesium Oxide						
1	2	P	S D	S D	D[a]	L[a]
2	0	P	P	P	S D	L[a]
3	00	P	P	P	S D	L[a]
4	000	P	P	P	P	D
Silica Gel						
1	2	S D[a]	D[a]	L[a]	L[a]	L[a]
2	1	P	S D	D[a]	L[a]	L[a]
3	0	P	S D	S D	D[a]	L[a]
4	00	P	P	S D	S D	L[a]
5	00	P	P	S D	S D	L[a]
Tribasic Calcium Phosphate						
1	2	S D	D[a]	L[a]	L[a]	L[a]
2	1	P	S D[a]	L[a]	L[a]	L[a]
3	0	P	S D[a]	D[a]	D[a]	L[a]
4	00	P	P	S D	D	D[a]
5	00	P	P	P	S D	D[a]

[a] Capsules became brittle and cracked.

TABLE VI.—PRESCRIPTION NO. 3 WITH INERT POWDERS

(Capsules Stores in Closed Containers)

Inert Powder, Grains	Size of Capsule	Time in Days: 0	1	2	4	14
Magnesium Carbonate						
1	2	P[a]	P[a]	P[a]	P[a]	P[a]
2	1	P	P	P	P	P
3	00	P	P	P	P	P
4	000	P	P	P	P	P
Light Magnesium Oxide						
1	2	P	P	P	P	P
2	0	P	P	P	P	P
3	00	P	P	P	P	P
4	000	P	P	P	P	P
Silica Gel						
1	2	S D[a]	S D[a]	S D[a]	S D[a]	S D[a]
2	1	P	P	P	P	S D
3	0	P	P	P	P	S D
4	00	P	P	P	P	P
5	00	P	P	P	P	P
Tribasic Calcium Phosphate						
1	2	S D	P	P	P	P
2	1	P	P	P	P	P
3	0	P	P	P	P	P
4	00	P	P	P	P	P
5	00	P	P	P	P	P

[a] Capsules became brittle and cracked.

PRESCRIPTION NO. 4

℞ Sodium iodide
Sodium nitrite, *āā* gr. iiss

Misce et ft. caps. no. xx.
Sig.: One t. i. d. as directed.

This prescription contains two deliquescent drugs. When it was compounded as written, the contents of the capsules turned yellow in color and liquefied after standing two days in open containers. Capsules placed in closed containers developed a light yellow color after standing two weeks and smelled strongly of iodine, but the contents of the capsules remained dry.

The prescription was compounded using various amounts of inert powders. In all cases, the sodium

iodide was first triturated with the inert powder and the sodium nitrite was then added. The temperature during compounding was between 65° and 67° F.; the relative humidity was 21–24%.

The results on the capsules stored in open containers were as follows:

Magnesium Carbonate.—Capsules varying in size from 2 to 000 and containing from 1 to 5 grains of magnesium carbonate per individual dose, although white powders when compounded, turned yellow after several days of standing. Although the absorbent powder prevented liquefaction in most cases, the gelatin capsule itself became soft and sticky.

Light Magnesium Oxide.—Capsules varying in size from 2 to 000 and containing from 1 to 4 grains of light magnesium oxide per individual dose, although white powders when compounded, turned yellow on standing. With small quantities of the absorbent, liquefaction occurred; with larger quantities of the absorbent, the contents of the capsules remained dry, but the gelatin capsule itself became soft and soggy.

Silica Gel.—Capsules varying in size from 3 to 00 and containing 1 to 5 grains of silica gel per individual dose, although white or faintly yellow when compounded, discolored rapidly and resulted in a black-colored capsule on standing two weeks. With 1 grain, 2 grains and 3 grains of silica gel per capsule the contents of the capsules became liquid at the end of two weeks' standing. When 4 or 5 grains of silica gel was added per capsule, the contents of the capsules were powders at the end of two weeks, but there was black discoloration and the capsules themselves were soft and soggy.

Tribasic Calcium Phosphate.—Capsules varying in size from 3 to 00 and containing from 1 to 5 grains of tribasic calcium phosphate per individual dose, although white powders when compounded, discolored rapidly on standing for two weeks, becoming either yellow liquids or yellow-speckled powders contained in soft, soggy capsules.

The results on capsules stored in closed containers were as follows:

Magnesium Carbonate, Silica Gel and Tribasic Calcium Phosphate.—Capsules containing up to 5 grains of the absorbents per individual dose turned yellow and smelled strongly of iodine after standing a few days. The contents remained dry over a period of two weeks.

Light Magnesium Oxide.—Capsules varying in size from 2 to 000 and containing 1 to 4 grains of light magnesium oxide per individual dose discolored only slightly after two weeks' standing. Capsules containing 4 grains of light magnesium oxide per individual dose remained white powders after the two-week standing period. It is possible that the discoloration was retarded or entirely prevented in the case of light magnesium oxide because magnesium oxide will take up moisture to form magnesium hydroxide, which in turn is capable of neutralizing the traces of free nitrous acid liberated during the decomposition of sodium nitrite. The neutralized nitrous acid is incapable of liberating iodine from sodium iodide, and hence discoloration is prevented.

In summarizing the effects of inert powders on prescription No. 4, it may be said that none of the inert powders prevent discoloration and deterioration of capsules stored in open containers. The prescription can be satisfactorily compounded using one or more grains of light magnesium oxide per capsule and dispensing in a closed capsule vial.

DISCUSSION OF RESULTS

In a study of the effects of several inert powders in preventing liquefaction of capsules due to the formation of an eutectic mixture, light magnesium oxide and magnesium carbonate, both of which are light, fluffy powders, were most effective. This was also the conclusion reached by Husa and Becker (1). Silica gel and tribasic calcium phosphate are both effective in preventing liquefaction in some cases, especially when the capsules are stored in closed containers. Nevertheless they are inferior to light magnesium oxide and magnesium carbonate as inert powders. One explanation for this may be that both silica gel and tribasic calcium phosphate are heavier powders than light magnesium oxide and magnesium carbonate, hence not as bulky. Silica gel and tribasic calcium phosphate are not effective in preventing the formation of cement-like masses in capsules.

The results show that liquefaction of capsules containing deliquescent ingredients may be prevented in most cases by the addition of an inert powder and by storage in closed capsule vials. As to relative efficiency of the various powders studied, light magnesium oxide and magnesium carbonate were best, tribasic calcium phosphate ranked next, and silica gel was poorest.

Deterioration and discoloration of capsules containing sodium iodide and sodium nitrite in equal parts is most effectively prevented by the addition of light magnesium oxide as an inert powder and by storage in closed capsule vials. Magnesium carbonate, silica gel and tribasic calcium phosphate are not effective.

SUMMARY

A study was made of the efficiency of silica gel and tribasic calcium phosphate as compared with light magnesium oxide and magnesium carbonate when used as inert powders in capsules that liquefy due to deliquescence or the formation of an eutectic mixture. In general, both light magnesium oxide and magnesium carbonate are superior to silica gel and tribasic calcium phosphate as inert ingredients in capsules.

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The Nontoxicity of Levulinic Acid*†

By R. G. Tischer, C. R. Fellers and B. J. Doyle

The discovery of a practical method for the preparation of levulinic acid (β-acetylpropionic acid) from corn at a very low cost has resulted in consideration of this acid as an element in the production and preservation of foods. Experiments conducted in this laboratory show that levulinic acid may be used successfully as an acidulant for carbonated and fruit beverages, jams, jellies and mayonnaise. Before industrial use could be made of this acid, it was necessary to obtain information as to its action in the human body. On the basis of chemical structure it should act similarly to the well-known acetic and propionic acids. A very complete bibliography of 423 references covering levulinic acid and its salts has just been compiled by the Division of Research Development of the A. E. Staley Manufacturing Company (1).

The literature reveals only a few references of a physiological nature. Gisselsson and Sylvan (2), in self-experiments using the calcium salts of gluconic and levulinic acids, caused a slight acidosis by the administration of massive doses of these salts.

Gordon, Kough and Proskouriakoff (3) have concluded that calcium levulinate administered orally, intravenously or subcutaneously is nontoxic for rats and human tuberculosis patients.

Greville and Dodds (4) injected intravenously 20 cc. of a 25% solution of calcium levulinate. Their subjects were one normal person, four tetany patients, and five jaundiced patients (preoperative treatment). No reactions whatsoever were observed.

Proskouriakoff (5) states: "Calcium levulinate was thoroughly tested by us on animals, and its extensive trial on tuberculosis patients showed no untoward symptoms .... As the low toxicity of levulinic acid in its salts was well established by previous investigators (Weintraud, W., *Arch. exptl. Path. Pharmakol.*, 34, p. 367), as well as by our own experiments on animals and men, it seemed to be desirable to prepare and try the levulinates of some other metals, the inorganic salts of which were tested and were shown to have some therapeutic value."

Although these reports indicate the nontoxicity of levulinates, and in particular that of calcium, there seems to exist no definite proof of the nontoxicity of levulinic acid itself. It was to answer this question that the present investigation was undertaken.

EXPERIMENTAL

Animal Experiments.—Preliminary toxicity tests on rats were used to determine the advisability of further experiments with human subjects. Three groups of rats (three in each group) were fed 0, 1 and 2% of the diet as levulinic acid for a period of 16 days. The acid was thoroughly mixed with a full nutrient ration and fed without restriction of amount. The results are presented in Table I and show no indication of toxicity in the concentration used.

Guinea pigs and chicks were also used to investigate the toxicity of levulinic acid. The acid was fed to guinea pigs in graded amounts varying from 0.5 to 5.0 cc. of 10% levulinic acid per day by means of a 1-cc. pipette or a stomach tube. Autopsy findings on these animals (after etherization) indicated no change in the appearance of the internal organs. The chicks survived similar treatment without apparent change.

Experiments with Human Subjects.—All the tests used in this investigation are standard procedures, details of which are found in "Approved Laboratory Technique" by Kolmer and Boerner (6).

Blood sugar, non-protein nitrogen, and creatinine were determined by the Folin-Wu technique. The carbon dioxide combining power of the plasma was determined by the Van Slyke and Cullen method, using a Van Slyke gas analysis apparatus.

In the urinalysis, acidity was determined using alk-acid paper (Fisher Scientific Company); sugar was determined by Benedict's qualitative test and albumin by Roberts' qualitative test.

The six normal male adults selected for the test ingested 3 cc. of pure levulinic acid daily for a period of 30 days, Sundays excluded. The acid was diluted with 150 to 400 cc. of fruit juice to disguise the acid taste.

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Contribution 430, Massachusetts Agricultural Experiment Station.

† The levulinic acid used in these experiments was kindly supplied by the A. E. Staley Manufacturing Company, Decatur, Ill.

TABLE I.—EFFECT OF LEVULINIC ACID ON GROWTH RATE OF RATS (16 DAYS)

Number of Rat	Levulinic Acid in Feed, %	Original Weight, Gm.	Final Weight, Gm.	Total Gain in Weight, Gm.	Total Food, Gm.	Gain in Weight per Gram of Food, Gm.
1	0	90	125	35	230	0.152
2	0	120	175	55	250	0.220
3	0	106	144	38	264	0.144
4	1	96	140	44	205	0.215
5	1	88	143	55	211	0.260
6	1	88	148	60	232	0.259
7	2	100	190	90	292	0.316
8	2	112	202	90	306	0.294
9	2	104	193	89	275	0.324

TABLE II.—RESULTS OF THE BLOOD CHEMISTRY TESTS ON HUMAN SUBJECTS

Subject	Test No.[a]	Sugar, Mg./100 Cc.	Non-Protein Nitrogen, Mg./100 Cc.	Creatinine, Mg./100 Cc.	CO_2 Combining Power, Vol. %
R.G.T.	1	79.7	28.5	1.64	62.0
	2	80.5	30.1	1.59	66.0
	3	71.4	26.3	1.46	65.7
	4	81.2	29.4	1.57	70.0
C.R.F.	1	94.9[b]	31.3	1.69	65.0
	2	83.8	30.8	1.74	63.1
	3	68.2	26.3	1.70	61.8
	4	85.1	29.5	1.66	64.5
R.S.L.	1	81.9	32.7	1.14	59.4
	2	84.0	30.9	1.23	60.2
	3	64.1	27.2	1.19	60.1
	4	85.0	31.0	1.25	58.5
J.J.P.	1	79.4	31.7	2.00	67.1
	2	70.4	33.1	2.70	65.0
	4	75.2	30.0	1.86	69.4
A.S.L.	1	76.3	27.9	1.58	71.2
	2	73.5	30.0	1.24	69.0
	4	78.5	26.3	1.49	67.1
W.B.E.	1	83.0	26.9	1.23	73.0
	2	81.2	28.0	1.27	70.2
	4	84.5	29.3	1.29	70.5

[a] The dates on which the tests were made were: No. 1, October 30, 1941; No. 2, November 13; No. 3, November 13 (about 40 min. after test No. 2 and 30 min. after taking 3 cc. of levulinic acid in 200 cc. of water); No. 4, November 27. All tests were made at about 8:00 a. m.

[b] A small bowl of oatmeal with sugar was eaten 1 hr. before the test.

The control medical and laboratory examinations were made on all the subjects before the administration of the acid was begun. The same tests were repeated after two weeks and four weeks.

At the end of the two-week period, all the tests were repeated on three of the subjects 30 to 50 min. after the ingestion of 3 cc. of levulinic acid in 200 cc. of water. This extra test was included to discover any possible immediate effects of the acid.

Blood samples were taken at about 8:00 a. m., the subjects having fasted since the previous night. The urine sample was collected on the morning of the test. Two 10-cc. samples of blood were taken, one from each arm. One sample was mixed with lithium oxalate to prevent coagulation and was then used in the preparation of the Folin–Wu protein-free filtrate for determination of blood sugar, non-protein nitrogen and creatinine. The remaining 10-cc. sample was used in determining the carbon dioxide combining power of the plasma.

RESULTS

The blood sugar results shown in Table II may appear to be rather low unless it is remembered that all determinations were made on fasting samples. As the first tests were made before the administration of acid was begun, these values are considered to be normal for each individual.

All of the values included in Table II appear to be in close agreement with the normal values expressed in test No. 1 except in the three cases where an additional test (No. 3) was made about 40 min. after ingestion of the daily dose of 3 cc. of acid. In these tests, the blood sugar, non-protein nitrogen and creatinine decreased a little in all three subjects, but not to an extent that would indicate a pathological condition. As tests No. 2 and No. 4 were performed less than 24 hrs. after the preceding dose of acid, the normal results indicate a complete recovery from the depressing effects noted in test No. 3.

There was no depressing effect whatever on the carbon dioxide combining power of the blood even

when the blood sample was taken within one hour of the time of ingestion of 3 cc. of pure levulinic acid in water. Likewise the non-protein nitrogen and creatinine values are all well within the normal range and show nothing of physiological significance.

The results of the urinalyses are recorded in Table III. The tests for sugar and for albumin were negative. Color, *p*H and specific gravity did not show significant variation in any of the tests.

TABLE III.—RESULTS OF URINALYSES

Subject	Test Number	*p*H	Specific Gravity
R.G.T.	1	5.0	1.012
	2	5.0	1.016
	3	5.0	1.023
	4	7.5	1.020
C.R.F.	1	5.0	1.024
	2	5.0	1.031
	3	5.0	1.032
	4	5.0	1.018
R. S. L.	1	4.0	1.024
	2	5.0	1.031
	3	6.0	1.030
	4	5.0	1.025
J. J. P.	1	4.0	1.030
	2	5.0	1.030
	4	5.0	1.030
A. S. L.	1	4.0	1.020
	2	6.0	1.030
	4	5.0	1.030
W.B.E.	1	4.0	1.012
	2	6.0	1.016
	4	5.5	1.014

The physical examinations of the men were performed in accordance with accepted medical practice. The results shown in Table IV indicate no significant variations in any of the measurements. The condition of the heart was observed by means of stethoscopic examinations, while "symptoms" were observed as general variation from normal health. Both were negative in every case. Careful and complete personal health diaries for each subject were kept before, during and following the period of this experiment. There was nothing of physiological significance in these data.

DISCUSSION

Of the three sets of data tabulated in Table IV, variation from normal conditions is observed only in the biochemical tests made on the blood drawn within one-half hour after the sample of acid was taken. Although a depression of the blood sugar level, non-protein nitrogen and creatinine is evident in these cases, these are not considered significant deviations from the normal because all other tests indicate the ability of the body mechanism to overcome the effect of the acid quickly and completely in every case. The absence of change in the alkali reserve balance as shown by the carbon dioxide combining power of the plasma only adds further weight to the evidence that the levulinic acid, at the level used in these tests, has only an insignificant effect, if any, on the human system.

CONCLUSIONS

1. No significant changes in the blood and urine, nor in general physical well-being resulted from the administration to men of 3 cc. of levulinic acid per day for 30 days.

2. The immediate effects on the sugar, non-protein nitrogen and creatinine content of the blood are well within the normal variations which might accompany the ingestion of ordinary foods.

3. No cumulative toxic effects are indicated.

TABLE IV.—MEDICAL EXAMINATION DATA

Subject	Age	Test Number	Weight, Lb.	Blood Pressure, Mm.	Pulse per Min.
R.G.T.	29	1	161	128/80	72
		2	165	138/78	72
		4	168	130/80	78
C.R.F.	47	1	169	136/84	66
		2	170	132/88	66
		4	172	132/88	72
R. S. L.	26	1	210	122/84	90
		2	214	128/86	84
		4	214	126/84	90
J. J. P.	23	1	117	110/70	78
		2	115	118/68	84
		4	116	116/70	74
A. S. L.	28	1	160	104/60	60
		2	159	108/66	48
		4	161	114/68	60
W.B.E.	29	1	164	138/88	72
		2	165	136/84	72
		4	165	130/84	66

4. Laboratory tests on albino rats, guinea pigs and chicks show that levulinic acid is nontoxic to these animals when fed to the extent of 5% of the food intake.

5. These preliminary studies suggest that levulinic acid in small amounts may be used safely to acidulate foods or beverages.

We wish to express our appreciation to G. E. Gage, Head of the Department of Physiology of the Massachusetts State College, for aid in interpreting the data and to C. W. Truehart, Medical Technologist of the Northampton State Hospital, for performing the clinical laboratory tests.

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Nomenclature Confusion in the Case of the Balsam Poplar or Tacamahac*†

By Kenneth Redman‡

Populus balsamifera Linné was the binomial invariably applied to the Balsam Poplar or Tacamahac until Farwell (1) took exception to the name in 1919, and said that "the binomial. . . belongs to the Carolina Poplar, as usually understood. . . ." The confusion in the literature regarding the taxonomical status of these poplars following Farwell's article seems to be due in part, at least, to Linné's error in botanical synonyms for *P. balsamifera* in his "Species Plantarum" (2). In this connection, Farwell (3) has pointed out that the technical description of Linné is from the "Hortus Cliffortianus" (4), and that ". . . reference to the latter publication shows that species No. 4, *Populus foliis cordatis crenatis*, is the one referred to. This is founded solely on *Populus nigra, folio maximo, gemmis balsamum odoratissimum fundentibus* Catesby (5), . . . a Carolina species. There is therefore no question . . . that the binomial *Populus balsamifera* belongs to the Carolina Poplar, as usually understood, since in the last analysis the Linnæan species is founded upon that of Catesby."

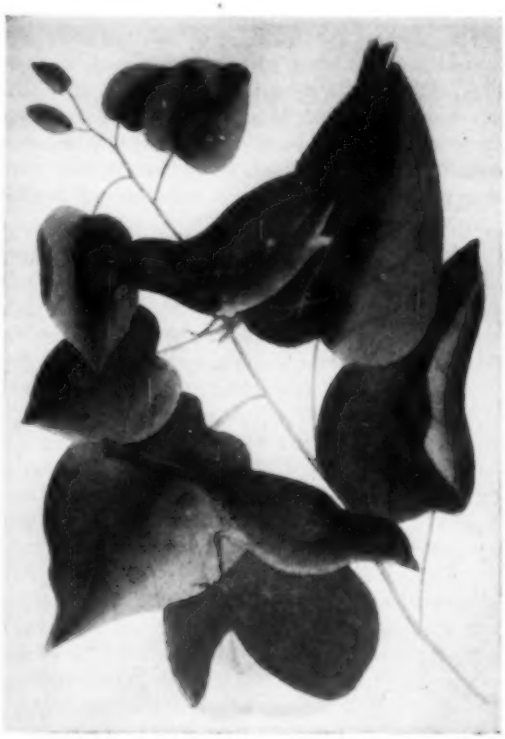

Fig. 1.—Catesby, M., "The Natural History of Carolina, Florida and the Bahama Islands," 1731, t. 34.

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Presented to the Scientific Section of the A. PH. A., Detroit meeting, 1941.

† This paper is based on part of a thesis presented to the Graduate School of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

‡ The writer is indebted to Professor W. O. Richtmann, Department of Pharmacognosy, University of Wisconsin, for directing this work.

Sargent (6) accepted Farwell's view after examining a photograph of Catesby's specimen (Fig. 1) from which his figure was made. Wiegand and Eames (7) and Stout (8) also accept this view.

House (9) comments on Catesby's citation as follows: "The reference of Catesby which Sargent picks as the type of *Populus balsamifera* Linné is the third citation given by Linnæus. . . . The first citation from the 'Hortus Cliffortianus' is positively referable to our northern poplar, it is the tree known to Linnæus. . . ." House (10), however, considered that Linné erred in referring Catesby's plate to *P. balsamifera*, but does not think this is sufficient reason "to reject the Linnean name for the northern poplar or to connect the name 'balsamifera' with a tree of such doubtful specific value as possessed by *P. angulata* Michx. f. (considered as a botanical synonym for the Northern Balsam Poplar by Sargent (11))." It is true, as House claims, that Catesby's reference (12) which Sargent (13) and Farwell (14) pick as the type of *P. balsamifera* Linné is the third citation given by Linné in his "Species Plantarum" (15), but it has already been shown that Farwell pointed out that the technical description of Linné is referable to Catesby's description. This is also shown by the fact that the citation in the "Species Plantarum" (16) to Royen (17) refers to "POPULUS foliis cordatis crenatis" in the "Hortus Cliffortianus" (18). Also, Royen (19) gives the citation of Catesby (20), *Populus nigra, folio maximo, gemmis Balsamum odoratissimum fundentibus*, as a botanical synonym.

It is not entirely clear what House (21) meant by the words ". . . the first citation from the 'Hortus Cliffortianus' is positively referable to our northern poplar. . . ." If we place a comma after *citation*, the "Species Plantarum" (22) is referred to, which fits in nicely with reference to the "third citation" (23), but if we interpret the wording literally, the "first citation" could either refer to the primary citation under species No. 4, or to species No. 1 of the "Hortus Cliffortianus" (24). Since the last two possibilities have already been discussed, species No. 1 of the "Hortus Cliffortianus" (25) remains for consideration. "1. POPULUS foliis subrotundis dentato-angulatis: subtus tomentosus (;) Populus alba. Dod. Dalech. hist. 87. (;) Populus alba Leuce. Bauh. hist. (;) Populus alba vulgo Albarus. Caes. Syst. 120. (;) Populus alba, majoribus foliis. Bauhin pin. 429. Boerh. (;) Populus alba, minoribus foliis. C. B. 42 lugdb. 2, p. 211."

Here it may readily be seen that Linné's descriptive name in the first citation clearly describes the leaf of *Populus alba*. In addition, all of the remaining citations have *alba* for what we now recognize as the specific name. Further, the fifth citation, *Populus alba, majoribus foliis* Bauhin (26), is referred by Miller (27) to the White Poplar or *Abele-tree*. Thus it appears that House's exception to Sargent's (and Farwell's) view that the binomial *Populus balsamifera* Linné belongs to the Carolina Poplar is not valid.

The first botanical synonym for *Populus balsamifera* in the "Species Plantarum" (28) "Populus foliis cordatis crenatis basi nudis petiolis teretibus," Wachendorff (29), has not been commented on in the literature consulted, but reference to this citation (Fig. 2) shows that "POPULUS foliis cordatis, crenatis" in the "Hortus Cliffortianus" (30) is given as a botanical synonym, which has already been discussed.

The fourth citation under *Populus balsamifera* Linné in the "Species Plantarum" (31), "Populus foliis ovatis acutis serratis," Gmelin (32), is taken as the type for *P. balsamifera* by Sudworth (33) when he says "Gmelin's plate 33, cited by Linnæus, clearly represents the foliage of our balsam poplar and in no way the deltoid foliage of our eastern cotton-wood. The writer is, therefore, here retaining *Populus balsamifera* Linné for the balsam poplar."

Upon inspecting Gmelin's "Flora Siberica" (34), we find he refers his "Populus foliis ovatis, acutis serratis" to "Populus foliis cordatis crenatis" Linné (35), and "Populo simili arbori resinosæ, altera" Bauhin (36). Again referring to the "Hortus Cliffortianus" (37), "POPULUS foliis cordatis crenatis" is the one referred to.

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826. *POPULUS. Linn. gen.* 909.

1. POPULUS foliis fubrotundis, dentato-angulatis, utrinque glabris. LINN. HORT.

A. STAMINEA.

B. PISTILLATA.

2. POPULUS foliis fubrotundis, dentato-angulatis; fubtus tomentofis. LINN. HORT.

A. STAMINEA.

B. PISTILLATA.

3. POPULUS foliis deltoïdibus, acuminatis, ferratis. LINN. HORT.

A. STAMINEA.

B. PISTILLATA.

4. POPULUS foliis cordatis, crenatis: bafi nudis: petiolis teretibus.
POPULUS foliis cordatis, crenatis. LINN. HORT.

5. POPULUS foliis cordatis, obfolete ferratis, infimis ferraturis glandulofis: petiolis lateraliter utrinque planis.

827. *JUNIPERUS. Linn. gen.* 917.

1. JUNIPERUS foliis feffilibus, patentibus. ROY. LEYD.

A. STAMINEA.

B. PISTILLATA.

Fig. 2.—Van Wachendorff, E. I., "Horti Ultraiectin Index," 1747, p. 294.

But Gmelin's second synonym, "Populo simili arboræ resinosæ, alteri" Bauhin (38), evidently refers to "Arbor Populo similis resinosa altera." A free translation of Bauhin's (39) remarks concerning this tree is to the effect that "The Tacamahaca collected in New Spain is from an odorous tree, as large as the poplar and whose fruit is the color of a peony." It seems then that Gmelin erred in his synonymy, in the first instance referring to the Carolina Poplar and in the second instance, to a tree that is not a poplar at all. Gmelin's illustration (40), however, could apply to the Balsam Poplar, which is the view taken by Sudworth (41).

CONCLUSION

It has been shown that Linné in all probability erred in his citations for *Populus balsamifera* in his "Species Plantarum," for while the first and second citations are referable to the third, Catesby's Carolina Poplar, it is possible that the illustration of the fourth citation is referable to our Northern Balsam Poplar, which was commonly known as *Populus balsamifera* Linné for over a century and a half, and which Sudworth selects as the type. That Linné's *Populus balsamifera* was a composite can also be seen by the fact that the primary citations in his "Species Plantarum" and "Hortus Cliffortianus" are so different that they could hardly be considered to refer to the same tree. Thus, although *Populus balsamifera* is a *nomen confusum*, the preponderance of Linné's citations are referable to *Populus nigra, folio maximo, gemmis Balsamum odoratissimum fundentibus* Catesby (42), which is the type selected by Farwell, Sargent, Stout and Rehder (43). The oldest valid name for the Balsam Poplar, or Tacamahac, is *Populus tacamahacca* Miller (44).

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Book Reviews

Textbook of Clinical Parasitology, by DAVID L. BELDING, M.D., Professor of Bacteriology and Experimental Pathology, Boston University School of Medicine, Member of staff of Evans Memorial, Massachusetts Memorial Hospitals. D. Appleton-Century Co., Inc., New York, N. Y., 1942. xxi + 888 pp., 279 figs., 44 tables and 4 colored plates. Price, $8.50.

Knowledge of the diagnosis and treatment of parasitological infections is very important and must receive more attention in the present conflict, since many theaters of war are located in the tropical and subtropical countries. This book is intended for medical students, physicians, public health officials, medical personnel in the armed forces, laboratory workers and biologists. The book is written from the standpoint of one responsible for teaching the subject, and to accomplish this end, numerous illustrations are used throughout the text. Many of the illustrations are diagrammatic and convey a fairly complete picture of the topic under discussion, independent of the text. Numerous keys are also incorporated in the text which should prove helpful to those who have need for a ready reference book. In place of the more popular terms, the author employs a few expressions which are little used to-day, such as alexin-fixation instead of complement-fixation. Nearly every chapter is accompanied by a few selected references, which should enhance the usefulness of the book for those desiring more than an elementary knowledge of the subject. The book, as the title implies, is a textbook and in this respect it fulfills its purpose.—H. E. Morton.

Synopsis of Materia Medica, Toxicology and Pharmacology, by FORREST RAMON DAVISON, Medical Department, The Upjohn Company. Second edition. The C. V. Mosby Company, St. Louis, Mo., 1942. 695 pp., 45 figs., 12.5 x 19.5 cm. Price, $5.75.

The writing of a synopsis in any field entails the careful selection of material to be included. Dr. Davison has not always been wise in his choice of subject matter, for at the expense of much valuable information, such drugs of secondary importance as conium, the sulfone hypnotics, and new agents which are almost untried are discussed, while many important facts are omitted. For example, there is nothing stated about the mode of destruction of epinephrine, the value of the short and intermediate acting barbiturates in cocaine poisoning, nor the more modern treatment of acute and chronic alcoholism. It is pleasing, however, to see the inclusion of such modern findings as the use of dihydrotachysterol to replace parathormone.

This synopsis is divided into two parts. Part I, comprising 95 pages, is given over to the consideration of fundamental principles of pharmacology, materia medica and prescription writing. Pharmacists will be pleased to see in this book many helpful suggestions regarding prescription writing, made available in such a way as to encourage the physician to prescribe in a simple and straightforward manner. Particularly noteworthy is the section on vehicles in which the most useful properties of coloring and flavoring agents are tabulated and the basic principles of disguising medicaments are outlined.

Part II develops the basic principles of the pharmacology of individual drugs, divided into the following sections: drugs acting on the skin and mucous membranes, drugs acting locally on the gastrointestinal tract, drugs acting on the central nervous system, drugs acting on the peripheral nervous system, drugs acting on the muscles, diuretics, metals and metalloids, and the sulfonamides. Ideal methods, which are all-embracing, for classifying the agents commonly used in therapeutics are difficult to attain. In broad outline, Davison has developed a convenient classification scheme. Unfortunately in the organization of subheadings the scheme frequently fails, creating unnecessary overlapping and misplacements. The chapter on antiseptics is a case in point. The classification is loose, the scheme being partially chemical and partially therapeutic, a scheme which has never been satisfactory. Further, the subsection on intestinal disinfection which appears in the chapter on gastrointestinal drugs, in the opinion of the reviewer, belongs more properly in the antiseptic chapter. There are many other chapters which show such poor organization. The section on anthelmintics has no organization whatever.

The chapters on autonomic pharmacology are especially well written. This is important, for since the discovery of the true nature of acetylcholine, a clear understanding of this phase of pharmacology is essential for the comprehension of many problems. The tracings and the charts in this section contribute much in the interest of clarity.

It is gratifying to see so many practical prescriptions scattered throughout the book. It is unfortunate that many of these prescriptions are misplaced. A prescription for methyl salicylate, to be used for counterirritation, is placed under the central action of the salicylates; in the section on counterirritation appears a prescription using alcohol to lessen sweating; in the section on antiseptics is found a prescription to tolu to be used as an expectorant, and there are many other examples of such misplacement.

Dr. Davison's book contains many errors of fact. Some of these are relatively trivial, but some are serious. For example, the advocation of the use of metrazol in the treatment of morphine poisoning is positively dangerous in view of the long-known biphasic action of morphine. Since cocaine is a central stimulant, the use of camphor or strychnine in acute cocaine poisoning is also a highly questionable procedure.

This book can be recommended as a text for pharmacy students only if the instructor is fully conscious of the organizational defects and providing he is sufficiently well grounded in pharmacological fundamentals that he may point out its errors. For reference purposes, there are many books which are more suitable.

The press work in this book is very poor, there being many examples of broken type and crowded spacing. The rhetoric leaves much to be desired. For example, in referring to ipral sodium the author states that "its actions and uses are the same as those of calcium," although it is obvious that calcium ipral, and not calcium, is meant.—M. W. GREEN

A Textbook of Dental Pharmacology, Materia Dentica and Pharmaco-Therapeutics, by WILLIAM H. O. MCGEHEE, M.D., D.D.S., F.A.C.D., and MELVIN W. GREEN, Ph.G., B.S., Ph.D. Second edition. The Blakiston Company, Philadelphia, Pa., 1941. xiv + 462 pp., 15 x 23 cm. Price, $5.00.

The scope and quality of this text has been greatly increased in the present edition. Aside from the pharmacological and therapeutic considerations, the book contains brief but well-written chapters on pharmacognosy, pharmacy and prescription writing. Mastery of this portion of the book by the dental student would do much toward giving him the confidence and knowledge necessary for the proper employment of prescription writing in his profession.

The appendix is well organized, containing toxicological summaries, tabular information on solubilities, metrological equivalents and percentage solutions. Of particular interest to pharmacists as well as dentists is the dental formulary and prescription guide. The authors acknowledge a great many sources for these formulas and prescriptions and have conveniently presented them under seven headings, according to their use in dentistry.

The pharmacological text material has been extensively revised and modernized by emphasis on principles, inclusion of illustrative material, addition of chapters on sulfonamide drugs and vitamins. The brief, compact treatment in this text accorded to local and general anesthesia and autonomic drugs is excellent. Many of the fundamental and theoretical details have necessarily been omitted, but the omission has been rendered almost painless by the quality of the writing. Perhaps the most serious criticism in this respect is the extreme brevity with which normal physiological functions are treated before consideration of the effects produced by pharmacological modification of these functions. Considering the extent of the revision, very few organizational errors are apparent.

Representing as it does the combined experiencet of the authors as physician-dentist and pharmaciss-pharmacologist, the book should be found exceedingly useful for study and reference by the student or practitioner of dentistry or pharmacy.—L. W. HAZLETON.